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The entire human population is chronically exposed to various contaminants, including lead and chlorinated hydrocarbon pesticides. Studies were undertaken to assess the effects of chronic exposure to lead on the toxicities of two such pesticides, lindane and dieldrin.

Mice were given lead as the acetate in drinking water, at levels of 75, 150 or 300 ppm lead for eight weeks. The acute LD50 of lindane (98 mg/kg) given in corn oil by oral intubation was not altered significantly ($P > .05$) by administration of 150 ppm lead. Similarly, the acute LD50 of dieldrin (45 mg/kg), given in corn oil by oral intubation, was not changed significantly ($P > .05$) by the same lead exposure.

In subacute studies, lindane was given daily by oral intubation in corn oil at a level of 40 mg/kg for the final two weeks of lead exposure. The excretion of delta-aminolevulinic acid in the urine was increased in a dose-related fashion by lead but was unaffected by

lindane. There was no evidence of significant interaction between the two agents. Urinary coproporphyrin excretion was increased slightly by both agents, and when lindane was given to mice receiving 300 ppm lead, levels rose from a control urinary concentration of 0.21 microgram per ml to 0.37 microgram per ml ($P < .05$). Urinary excretion of uroporphyrin was not altered by either agent. Thus, lead and lindane apparently interacted in an additive fashion at at least one step in the heme synthesis pathway, and failed to interact at at least two steps.

Lead depressed blood hemoglobin concentration (from a control value of 13.5 grams per 100 ml to 12.8 grams per 100 ml), an effect predictable from the blockade of heme biosynthesis by the metal. Lindane did not affect hemoglobin concentration when given by itself but prevented the fall in concentration elicited by lead. Hematocrit was decreased in a dose-related fashion (from 43.7% to 41.1%) by lead, and lindane appeared to accentuate the decrease (40.0% when both were given). The agents were thus seen to interact at two or more sites, one of which was probably the heme biosynthetic pathway.

Levels of hepatic microsomal cytochrome P-450 were depressed in a dose-related fashion upon administration of lead. In mice receiving 300 ppm lead, levels were depressed to about 50% of the control level. This effect suggested the possibility that individuals exposed to lead may suffer impairment of oxidative metabolic pathways

dependent upon cytochrome P-450. This could have the effect of increasing the toxicity of a compound taken into the body, or of increasing the duration of the action of the compound. Lindane increased levels of cytochrome P-450 an average of 83% over respective counterparts not receiving lindane. This effect was probably related to an induction of hepatic microsomal enzyme systems. Lindane, at the level given, overcame the effects of lead on cytochrome P-450 levels.

Despite the effect of lead on cytochrome P-450, lead administration did not affect the length of time mice slept ($P > .05$) when given 40 mg/kg pentobarbital sodium. Lindane reduced sleep time from 16.4 minutes to 8.5 minutes ($P < .05$) whether or not lead was given. This effect was attributed to induction of hepatic microsomal pentobarbital-metabolizing enzymes.

Lead did not alter susceptibility to clonic seizures as determined by intravenous infusion of pentylenetetrazol at a constant rate. Lindane, however, increased by an average of 53% the amount of pentylenetetrazol necessary to induce seizures; when seizures occurred in lindane-treated mice they were often fatal in outcome, whereas mice not given lindane rarely died from this procedure. Lead appeared to raise mortality levels in this test. Dieldrin decreased seizure threshold an average of 31%. It was concluded that lindane and dieldrin elicit central stimulation by different mechanisms.

When seizure susceptibility was determined by the low-frequency electroshock test, lead appeared to increase susceptibility slightly, whereas lindane consistently decreased it to a significant extent (an average decrease of 23%) and dieldrin raised susceptibility (an average increase of 26%).

The length of time mice were able to maintain equilibrium on a rotating horizontally-oriented wooden rod was determined. Lead administration did not affect this parameter even when given in concentrations up to 4800 ppm for three months. Four weeks' administration of 0.5 or 1.0 ppm dieldrin in food impaired the ability of mice to remain on the rod. When lead administration was added to dieldrin feeding, the agents appeared to act antagonistically in this test. Thus, it appeared that this test is valid for assessing chronic dieldrin intoxication in mice, but that lead administration renders the results obtained from the test inconclusive.

It was concluded that the mouse is a useful laboratory animal in studies of the biochemical and hematologic effects of chronic lead poisoning, but is not useful in the assessment of central damage due to lead. If the findings of these studies can be extrapolated to humans, it may be concluded that although the lethality of lindane is unaffected by chronic administration of lead, an individual exposed to both agents suffers additive effects, such as was seen in the coproporphyrin study. Therefore, an individual exposed to lead should take special precaution

to avoid exposure to lindane, and an individual exposed to lindane should guard against exposure to lead.

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I. GENERAL INTRODUCTION

Lead intoxication has plagued mankind for centuries. Hippocrates appears to have been the first to diagnose the condition, recognizing it as the cause of saturnine gout (Aub, Fairhall, Minot and Reznikoff, 1925). Lead has been implicated as a factor in the downfall of the Roman Empire (Gilfillan, 1965). Its toxicity was recognized by many of the pioneers in the field of medicine: Dioscorides, Avicenna, Crato, Huxham, Burton, des Planches and Orfila, the last of whom included lead in his famous treatise on toxicology (Holmstedt and Liljestrand, 1963).

Lead is a contaminant of at least two fractions of the environment to which the entire population is chronically exposed, i. e., the atmosphere and the soil (Haley, 1966; Goldsmith and Hexter, 1967). Kehoe, Story, Cholak, Hubbard, Bambach and McNary (1940) state that the average intake of lead by humans is about 0. 32 milligram per day. The same workers report that the ingestion of one milligram per day for three and one-half years results in no measurable pathological change; two milligrams per day for one year results in increased elimination and greater retention of lead. It is therefore not surprising that lead is found in human tissues (Goldsmith et al., 1967; Thomas, Milmore, Heidbreder and Kogan, 1967). It has been

found in the blood and urine of people in Finland, Peru and New Guinea, and in the livers and kidneys of Europeans and South Africans (Copeman, 1945; Goldwater, 1967). In general, higher levels of lead have been reported in persons in urban areas than in those living in rural or uncivilized areas.

Some individuals are exposed to greater levels of lead than are others, because of certain special environmental conditions. Lead contaminates sea water, and is found in some ceramic glazes, in tobacco, beverages, food, some paints, some drinking waters, plaster, nipple shields, nipple ointments, crayons, insecticides, body powders, collapsible dispensing tubes, herbicides, fungicides, varnishes, oil pigments, putty, caulking compound, linoleum, rubber, jewelry, chinaware, and at least one facial ointment (Patterson, 1965; Harris and Elsea, 1967; McNiel and Reinhard, 1967; Parry, 1967; Srivastava and Varadi, 1968). It is found in high concentrations in the air where lead is heated to a high temperature, as when it is melted to manufacture items commercially or in the home (lead sinkers and bullets), or when used storage batteries are burned as a source of heat by primarily low-income families (Williams, Schulze, Rothchild, Brown and Smith, 1933; Gocher, 1945). It is found in high atmospheric concentrations along heavily-trafficked roadways (Brief, 1962; Konopinski and Upham, 1967). This last example is doubtless due to the inclusion of the antiknock compound tetraethyllead in gasoline (Thomas et al.,

1967).

Workers in some lead industries are exposed to greater amounts of atmospheric lead than the population in general (Gocher, 1945; Giel, Kleinfeld and Messite, 1956). One report showed that the concentration of lead in the air in a battery manufacturing plant ranged from 0.09 to 0.65 mg/cubic meter (Giel et al., 1956). Exposure to such concentrations of air-borne lead has produced numerous instances of lead poisoning. In 1943, Dreessen studied 766 employees of a storage battery plant, and found 177 of them exhibited signs of "early plumbism." Of this group, 168 were absorbing abnormally high amounts of lead, and nine showed insipient plumbism (see also Gocher, 1945; Cotter, 1946).

Atmospheric contamination would appear to be a more dangerous situation than contamination of some other parts of the environment, as, according to Mehani (1966), 39% to 47% of inhaled lead is retained, whereas Kehoe, Thamann and Cholak (1933) found that only about 10% of ingested lead is absorbed. Lead in the atmosphere consists either of vapors of elemental lead or of lead oxide dust (Rezin and Drinker, 1939; Halley, 1941; Daubenspeck, Tienson and Noyes, 1944). Hughes (1965) states that lead oxide is formed on the surface of the melt, and is thrown into the atmosphere when the molten lead is stirred or poured. It should be noted that Haley (1966) contends that lead-bearing particles issuing from automobiles are too large to be

absorbed in the respiratory tract.

Many reports are to be found in the literature regarding lead poisoning from tap water (Joliff, Heidrich, Cain and Ohlsen, 1959; Bacon, Froome, Gent, Cooke and Sowerby, 1967; Crawford and Morris, 1967; Parry, 1967; Reed and Tolley, 1967). The study by Joliff and associates (1959) indicates that where lead water pipes are still in use, intoxication can be largely prevented by taking water for consumption only after the water has run long enough to flush out the heavily-contaminated portion.

Lead pica is a disease of unknown etiology wherein an individual, nearly always a child, ingests substances high in lead content, such as paint, plaster, and even dirt. The condition is observed in a significant portion of young children; Barltrop (1966) indicates that 20% of all children experience pica some time during childhood (although it is not stated whether this includes only those with lead pica). Jacobziner and Raybin (1962) estimate that 24% of children with lead pica have "probable lead poisoning." Greengard (1966) states that pica is responsible for most of the pediatric cases of lead poisoning.

The differences in symptomatology between acute and chronic lead intoxication are well recognized. Acute poisoning is manifest as a metallic taste, abdominal pain, vomiting, black stools, oliguria, collapse, coma, paresthesias, muscle weakness, possible constipation

or diarrhea, and occasionally hemolytic anemia and hemoglobinuria (Dreisbach, 1963; Harvey, 1965). Chronic lead intoxication is the more difficult malady to detect. Aub et al. (1925) list 27 possible symptoms, ranging from "lesions found constantly" to "lesions which have been reported to occur occasionally in people with evidences of lead absorption or lesions possibly due to lead." More recently it has been shown that the three lesions which Aub listed as being "found constantly" are not appropriately placed in such a category. The three lesions are (1) the Burtonian line on the gums, (2) anemia, and (3) basophilic stippling of erythrocytes. Moeschlin (1965) states that the Burtonian line is seen in "most cases of severe poisoning." Red cell count (a measure of anemia) is variable, some workers reporting decreases, some reporting no change, and some reporting increases (Artz, 1941; Kehoe, 1943; Gocher, 1945; Giel et al., 1956; Horiuchi, Noma, Asano and Hashimoto, 1962). Stippling of red cells is sometimes absent, and may depend on the time of day at which the blood sample is taken (Lehmann, 1924; Duvoir, Dérobert and Hadengue, 1947). Symptoms usually associated with chronic lead poisoning include the following: weakness, weight loss, constipation, colic, anorexia, arthralgia (saturnine gout; see Ehrlich and Chokatos, 1966), lassitude, nausea, vomiting, insomnia, headache, metallic taste, and stiffness (Ashe, 1943). Clinical signs include the Burtonian gum line, pyorrhea, malnutrition, hyperactive biceps and patellar reflexes,

tremor, pallor, sensory disturbances, myoedema, stupor, convulsions, delirium, coma, and increased density to X-radiation of the epiphyseal areas of the long bones in growing children (the so-called lead line) (Ashe, 1943). The increased density to X-radiation is sometimes absent in children early in chronic lead intoxication, and is sometimes present in apparently normal children (Sartain, Whitaker and Martin, 1964). Regarding the muscular weakness, the most-used muscles are affected first; thus, in a right-handed house painter, weakness of the right wrist would be noticed before weakness of the left wrist.

More recently, two more sensitive indicators of lead intoxication have been observed, i. e., increases in urinary levels of porphyrins and delta-aminolevulinic acid (ALA) (deMello, 1951; Byers, 1959; Joliff et al., 1959; deKretser and Waldron, 1963; Albahary, 1964; Chisolm, 1964; Kreimer-Birnbaum and Grinstein, 1965; Detection . . . , 1966). According to Albahary (1964), urinary delta-ALA increase is the most reliable test for lead intoxication. The pathogenesis of the increases in porphyrins has been concluded to be blockade of the incorporation of iron into protoporphyrin, thus giving rise to increased levels of heme precursors, specifically protoporphyrin, protoporphyrinogen III, coproporphyrinogen III, uroporphyrinogen III, and porphobilinogen; the increase in urinary delta-ALA appears to be due to the inhibition of the enzyme delta-ALA

dehydrase (Greengard, 1966). The concepts are illustrated in Figure 3-1.

The peripheral neuritis of lead poisoning is discussed by Brown and Smith (1937). Kostial and Vouk (1957) found that, in the cat, lead brought about a blockade of transmission in the superior cervical ganglion, and that less acetylcholine was released from the preganglionic site. They attributed this effect to either an inhibition of resynthesis of muscle phosphocreatine, which plays a role in acetylcholine metabolism, or to the inhibition of acetylcholine synthesis due to attachment of lead to the sulfhydryl group of coenzyme A, thus preventing the acetylation of coenzyme A, and subsequently, of choline (Nachmansohn and Lederer, 1939; del Castillo-Nicolau and Hufschmidt, 1951; Reisberg, 1954; Baier, 1958a). Sávay and Csillik (1958, 1959) have visualized myoneural synapses by means of lead.

With regard to the central nervous system, a few important symptoms are seen frequently. The young are especially susceptible to damage to the central nervous system due to lead (Greengard, 1966). There have been reports of mental retardation, although Gordon, King and Mackay (1967) found no correlation between the presence of Down's Syndrome (mongolism) and elevated lead levels in the blood (Thurston, Middlekamp and Mason, 1955; Lead . . . , 1964; Feigin, Shannon, Reynolds, Shapiro and Connelly, 1965; Perlstein

and Attala, 1966). Cerebral palsy and optic atrophy are reported as having been found as sequelae of lead poisoning in children (Perlstein et al., 1966). The occurrence of lead encephalopathy is noted by many workers (for instance, Popoff, Weinberg and Feigin, 1963; Feigin et al., 1965). Glotfelty (1942) reports a reversible psychosis in an adult.

In 1961, Cremer reported that the toxicity of triethyllead is greater than that of the tetraethyl derivative, but that the latter is transformed into triethyllead in the liver, both in vitro and in vivo. Harvey (1965) states that tetraethyllead is eventually converted to inorganic lead in the body. Toxic doses of tetraethyllead do not affect learning or memory in the rat, as measured by a water maze (Bullock, Wey, Zaia, Zarembok and Schroeder, 1966).

Several other effects of lead have been observed in experimental animals. Decreased I-131 uptake by rat thyroid was reported by Sandstead (1967). Schroeder, Vinton and Balassa (1963) found that lead shortened the life-span of the rat. Renal damage in rats and dogs, and renal tumors in rats have been observed (Finner and Calvery, 1939; Mao and Molnar, 1967). Bracken, Beaver and Randall (1958) noted the formation of intranuclear bodies. Lead is transferred through the milk of the rabbit to her young (Vahlquist and Sälde, 1943-44). Reproduction is impaired in rats, according to Dalldorf and Williams (1945). Malformations of embryos have been reported in the cases of

chicks and hamsters (Catizone and Gray, 1941; Ferm and Carpenter, 1967).

Lead appears to be handled in the body much like calcium (Aub, Fairhall, Minot and Reznikoff, 1926; Aub, 1935). This is not totally surprising, as, although the two elements are in neither the same period nor the same group in the periodic table of the elements, both are divalent cations. Increased urinary output of lead after exposure to light was reported by Pincussen (1933). Rapoport and Rubin (1941) found that cod liver oil and sunlight (but not heat alone) increased the toxicity of lead in rats, the effect being presumably due to increased absorption of the metal, which in turn was due to the presence of higher levels of vitamin D. Thus, as absorption of calcium is enhanced by increases in vitamin D levels, so also is the absorption of lead. The effect of vitamin D is so pronounced that the prevelance of lead poisoning in children increases during the months of increased exposure to sunlight (Rapoport et al., 1941; Jacobziner et al., 1962). After absorption, lead is carried in the blood in the form of di- or triphosphate, and is stored in the hard parts of bone, and in the fat cells of red marrow (Fairhall, 1943; Cooper, 1947).

The fate of intravenously-injected lead in rats was studied by Castellino and Aloj (1964). They found that 96% of the lead in the blood is bound to cellular elements, and that lead is distributed to the following organs, in order of decreasing levels of lead: kidney, liver,

bone, lung, spleen, heart, and muscle.

Lead has been shown to inhibit protein synthesis (Hass, Landerholm and Hemmens, 1967). Yaverbaum (1965) reports that increased serum aldolase activity correlates with urinary lead levels. In 1964 Waldron reported the failure of any change in serum aspartate and alanine transaminase levels to take place in humans exposed to lead, although blood levels of lead rose. Baier (1958b) found a decrease in arginase activity of human red cell hemolysates. Another effect reported is a decrease in capillary resistance in rats (Odeschalchi and Andreuzzi, 1959). It might be pointed out here that in very few studies of lead poisoning have mice been used as experimental animals.

In the mid-1940's, man began placing in his environment a new kind of pollutant -- the chlorinated hydrocarbon pesticides. Their advent was hailed as a major success in the biological sciences, and perhaps correctly so. However, it was soon realized that the effects of these substances on animal pests were also observed in humans; a compound which would kill a garden pest by central overstimulation might also kill the gardener by the same mechanism.

One such pesticide is lindane, the gamma isomer of 1, 2, 3, 4, 5,-6-hexachlorocyclohexane. Lindane has been found in the body fat of people in the United States, Britain, France, and India (Dale and

Quinby, 1963; Hayes and Dale, 1963; Hoffman, Fishbein and Andelman, 1964a, b; Dale, Copeland and Hayes, 1965; Egan, Goulding, Roburn and Tatton, 1965; West, 1967). Thus, it seems apparent that the population in general carries a body burden of lindane; in the United States this burden amounts to about 0.57 ppm in fat (Hoffman et al., 1964a).

Symptoms of lindane intoxication include vomiting, diarrhea, convulsions, dizziness, headache, tremors, muscular weakness, and irritation of eyes, nose and throat (Dreisbach, 1963). It will be noted that most of these symptoms are also symptoms of chronic lead intoxication. Depression of bone marrow function has been attributed to acute lindane intoxication (American Medical Association, 1962; Loge, 1965; West, 1967). Thus, one would see decreases in numbers of cellular elements in the blood, as well as a tendency for immature forms of these cells to be released into the blood.

An interesting set of symptoms was observed in laboratory animals by de Matteis, Prior and Rimington (1961) and by Ockner and Schmid (1961). They reported increases in levels of urinary delta-aminolevulinic acid and urinary porphyrins in rats, again symptoms seen also in chronic lead poisoning.

The insecticide dieldrin (any product containing not less than 85% 1, 2, 3, 4, 10, 10-hexachloro-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-6, 7-epoxy-1, 4-endo, exo, 5, 8-dimethanonaphthalein (Hunter, Robinson and

Richardson, 1963), has also been detected in human body fat (Dale et al., 1963; Egan et al., 1965; Hayes, 1965; Zavon, Hine and Parker, 1965). Since dieldrin is removed from the soil by food plants, it is logical that humans and other animals who consume these food plants would be found to contain the insecticide themselves (Mumma, Wheeler, Frear and Hamilton, 1966). Hunter, Robinson and Roberts (1969) found that the average daily per capita intake of dietary dieldrin ranges from that found in India, 1.6 micrograms, to that found in Italy, 24.3 micrograms. The average daily consumption in the United States was found to be 7.6 micrograms per person. Four classes of foods are reported to be responsible for nearly all the daily dietary intake of dieldrin. Approximately equal amounts of dieldren are consumed daily from animal tissues, animal products, cereals and breads, and vegetables (McGill and Robinson, 1968). Hoffman et al. (1964b) state that dieldrin levels in human body fat are about 0.11 ppm; this value is close to that given by Dale et al. (1963), 0.15 ppm. Robinson and Hunter (1966) place human fat levels slightly higher, at 0.22 ppm, ranging from 0.10 ppm to 0.73 ppm. The effects of acute dieldrin intoxication include those referable to hyperexcitation of the central nervous system: tremors, ataxia, and convulsions, followed by central depression, and finally death due to respiratory depression (Dreisbach, 1963). It will be noted that symptoms of intoxication with this agent also parallel those observed in lead intoxication. Hoffman, Adler, Fishbein and Bauer (1967) found no correlation between levels

of chlorinated hydrocarbon pesticides in human body fat and pathologic changes.

Since lindane and dieldrin elicit signs and symptoms of intoxication parallel to some extent to those of lead, the question arises as to the effects of combinations of lead intoxication and either lindane or dieldrin intoxication. Hardy (1966) points out that one must take into account "... the likelihood that lead in the body at levels considered, from industrial exposure, to be harmless can act with other factors to produce damage. If this is true, in judging what amount of lead is harmless for an individual or a population, other insults natural or man-made must be assessed." It would seem logical that the first agents one would want to investigate in assessing other insults might be those which elicit symptoms of intoxication similar to those of lead. Such agents are lindane and dieldrin; the former, because both hematologic and central effects resemble those produced by lead intoxication, and the latter because of the similarity of central effects to those elicited by lead. Thus, these could be two of the man-made insults which could have a distinct bearing on the level of lead harmful to man.

One of the purposes of the pesticide training grant, under which this work was done, is to define potential hazards with regard to pesticide exposure. Questions which research under this grant should seek to answer might include the following: What are the effects of

long-term exposure to low levels of a pesticide? Are there "no-effect" levels of pesticides? Are alterations in our environment necessary to sustain life in the face of increased contamination of the environment with pesticides and other foreign residues? And if so, what alterations need to be made, and how can these be made? What effect may other environmental contaminants have on the toxicities of pesticides?

The last question is related to the point raised by Hardy: " ... the likelihood that lead ... can act with other factors to produce damage." This is an important point, and demands investigation. Studies of individual stresses in the form of environmental contaminants are of great value; but the human organism is exposed to not just one environmental stress, but to many. The fewer stresses a study investigates, the more remote from the real situation the study is. In the field of environmental toxicology, one of the paramount concerns, then, would be in the area of multiple stress problems.

There has been a hesitancy on the part of investigators to undertake such projects, doubtless due to the difficulties inherent in such a study. It is obviously impossible to administer environmental contaminants to laboratory animals in a manner that accurately mimics the administration experienced by a human. But it seems better to at least attempt to approach a comparable regimen, and collect what data can be observed, than to make no attempt whatever to conduct studies in this area.

A purpose of toxicologic investigation is to define groups in the population who react in a particular fashion to chemical stresses. Some individuals are more tolerant of certain chemical substances than are others. Some are more sensitive to the side effects of certain therapeutic agents. This investigation was undertaken with the hope of defining a segment of the population who might be more susceptible to lindane or dieldrin intoxication than the rest of the population.

The primary purposes of this investigation are to determine the effects of chronic lead intoxication in mice, to determine the effects of the superimposition of chronic lead intoxication on either lindane or dieldrin intoxication and to consider the possibility that nominally innocuous levels of lead might actually prove harmful when superimposed on intoxication with either of these pesticides. At least a partial answer will be sought to the question, "Is it possible that some people are more sensitive to lindane or dieldrin because of exposure to another environmental contaminant, specifically, lead?"

II. GENERAL METHODS

Animals used were weanling male CF #1 mice, three weeks of age. The reason for using such young mice was the indication that the central nervous system of a young animal is more sensitive to damage by lead than that of an adult (Greengard, 1966). The mice were housed in groups of eight in plastic cages measuring approximately 27 cm x 16 cm x 10 cm. The animals were allowed free access to food and water at all times. Bedding was oven-dried wood chips.

Lead was administered as the acetate (trihydrate) salt, in drinking water, to which the mice had free access. It was necessary to add 0.1 ml glacial acetic acid per liter of tap water to dissolve the lead acetate. Except where otherwise indicated, lead acetate was given for eight-week periods. Concentrations in the data were expressed as those of lead ion.

Lindane (technical grade; obtained from Pennsalt, Inc.) was administered daily by oral intubation of a corn oil solution, at a dose of 40 mg/kg (about half the acute LD50) during the final two weeks of an eight-week administration of lead. This dose level resulted in few deaths over the two-week period. Controls (receiving corn oil only) died at about the same rate as lindane-treated mice.

Dieldrin (technical grade; obtained from Shell; lot # HI-1037), except where otherwise noted, was administered in the food. Purina

Lab Chow was soaked overnight in distilled water. An acetone solution of dieldrin of the proper concentration to yield 0.5, 1.0, or 2.5 ppm dieldrin (based on food weight before soaking) was then mixed with the Chow (concentrations obtained from Bone and Harr, 1966). This mixture was dried in small pieces in an oven at 32°C under a stream of air. The exact weight of the food, and therefore, the exact concentration of dieldrin in it, was not known after the food was dried, due to losses of food on the sides of containers and in the drying pans.

All comparisons between treated animals and control animals, except where otherwise indicated, were calculated by means of Student's _t_ test (Snedecor, 1956). Means were considered significantly different if and only if the value of _t_ was significant at the 95% level ($P < .05$).

III. GROSS TOXICITY STUDIES

Introduction

In general, signs and symptoms of intoxication are most readily seen when lethal or nearly lethal doses of an agent are given. Results of a study of gross toxicity give direction to further investigation, with regard to what parameters should be studied, and what parameters are likely not affected.

The most readily available information on additive toxicities is obtained from lethality studies. Such investigations are of a rather gross nature, but help to answer perhaps the most essential question in the clinical situation, "Is a patient poisoned by one or more agents likely to die as a result of this intoxication?" These studies were undertaken to assess the effect of chronic administration of lead on the acute lethalities of dieldrin and lindane, and the effect of acute administration of dieldrin on the acute toxicity of lead.

Methods

In studies involving chronic lead intoxication, lead was given in drinking water at a level of 150 ppm as the acetate for a period of eight weeks. In the study of acute lead intoxication, lead, as the acetate, was given intraperitoneally. Results were expressed as concentrations of lead ion. In studies of acute intoxication with

lindane and dieldrin, these agents were given orally in corn oil solution.

Values of LD50's were calculated by the method of Litchfield and Wilcoxon (1949). By this method, 95% confidence limits can be assigned to an LD50 and LD50's can be compared by means of a potency ratio. A slope function can also be calculated, which gives a rough estimate of whether or not two agents operate by the same mechanism in causing the observed effect. In all cases in this investigation, agents were compared at the 95% level of significance, and slope functions were compared at the same level of significance. In each experiment, four doses of the indicated intoxicant were administered to groups of eight mice each. Each experiment was performed at least twice. The LD50 is based on the number of deaths 48 hours after administration of the toxic agent.

Results

Chronic administration of lead for periods of up to six months, at levels of up to 4800 ppm lead caused little grossly observable change in appearance or behavior of the mice. The only symptom noted was diarrhea, seen when levels of lead greater than 600 ppm were administered. This effect was attributed to the saline cathartic action of the lead salt. Pilot studies carried out in the preparation of experiments for this thesis indicated that maximal effects of lead

poisoning on hematocrit and blood hemoglobin concentration occurred when 150 ppm to 300 ppm lead was given. Greater concentrations of lead did not lead to more severe depressions of hematocrit or blood hemoglobin concentration. The failure of more severe symptoms to occur upon administration of lead at higher levels was attributed to the cathartic action of the lead salt; i. e., catharsis interferred with lead absorption to a greater extent when higher levels of lead were given. For this reason, levels of 75 ppm, 150 ppm and 300 ppm were chosen for all the work in this series of studies.

When lead acetate was given acutely, by intraperitoneal injection, the only observable symptoms were those relating to central hyperexcitation, i. e., clonic and tonic seizures, and death due to respiratory paralysis, in tonic seizure. The median lethal dose of lead as the acetate was found to be 150 mg/kg, with 95% confidence limits of 116 and 194 mg/kg (Table 3-1).

Acute administration of lindane in lethal doses resulted in clonic seizures followed in some cases by tonic seizures and death due to respiratory paralysis. Deaths occurred from one hour postinjection to 25 hours thereafter. The median lethal dose was found to be 98 mg/kg, with 95% confidence limits of 80 and 120 mg/kg.

When lindane was given daily for two weeks by oral intubation, at a level of 40 mg/kg, central excitation was very evident. After a few days of such treatment the mice would become rather difficult to

Table 3-1. Acute lethalities of lead, lindane and dieldrin in CF #1 mice.

No.	Treatment	Route	LD50[a]	95% C. L.[b]	S[c]	P. R.[d]	S. R.[e]
1	Lead as the acetate	i. p.	150	116-194	1.59		
2	Lindane	p. o.	98	80-120	1.52		
3	Dieldrin	p. o.	45	27- 75	1.67		
4	Lead as the acetate + dieldrin (13 mg/kg)	i. p. p. o.	164	130-206	1.61	1.09	1.02
5	Lindane + chronic lead[f]	p. o. --	105	82-136	1.63	1.07	1.07
6	Dieldrin + chronic lead[f]	p. o. --	48	32- 72	1.97	1.07	1.18

[a] mg/kg.

[b] 95% Confidence Limits, mg/kg.

[c] Slope Function, calculated as $\frac{LD84/LD50 + LD50/LD16}{2}$.

[d] Potency Ratio, calculated as $LD50_1/LD50_2$ where $LD50_1 > LD50_2$ (to compare treatment 1 with treatment 4, 2 with 5, and 3 with 6).

[e] Slope Function Ratio, calculated as S_1/S_2, where $S_1 > S_2$ (to compare treatment 1 with treatment 4, 2 with 5, and 3 with 6).

[f] 150 ppm lead as the acetate in drinking water for eight weeks prior to lindane or dieldrin challenge.

handle, in that they seemed to be more "nervous", more "jumpy", were more difficult to capture for administration of drugs, and were more apt to attempt to bite the investigator.

Acute administration of dieldrin affected the mice in the same way as acute administration of lindane, as far as gross symptoms are concerned. Death was due, as with lindane, to respiratory paralysis. The median lethal dose was found to be 45 (27 to 75) mg/kg.

Chronic administration of lead at a level of 150 ppm for eight weeks did not alter the acute oral LD50 of lindane to a significant extent. The LD50's differed by only 7%, whereas a difference on the order of 40% would have been required to demonstrate statistical significance with these data. The slope functions differed by 7% (157% would have been significant with these data). This same lead exposure, likewise, did not seem to affect the acute oral LD50 of dieldrin. The difference in the two values of the LD50 would have to have been 91% for them to be significantly different; the actual difference was only 7%. The slope functions would have to have differed by 116% to be significantly different, but differed by only 18%.

Acute preadministration with one-fourth of the LD50 of dieldrin (13 mg/kg), a dose which resulted in no deaths by itself, did not affect the acute LD50 of lead to a statistically significant degree. The LD50 was raised 9% (40% was required for significance with these data) by dieldren pretreatment, and the slope function was increased by 2%

(75% was required for significance).

Discussion

The value of the LD50 of lead found here for mice, 150 mg/kg, is somewhat above the value given by Spector (1956) for lead (given intraperitoneally in the form of the acetate) in rats, 95 mg/kg. The same source also lists the LD50 of lead as the nitrate given intraperitoneally to rats as 169 mg/kg, and the LD50 of lead in the form of the monoxide given intraperitoneally to rats as 372 mg/kg. The LD50 of lead as the carbonate in guinea pigs is given as 96 mg/kg, when administered intraperitoneally. An extensive search failed to disclose any data on the acute lethal dose of lead acetate (or any other lead salt) in mice. But the present study indicates that the median lethal dose of lead as the acetate in mice is well within the range of the median lethal doses of other lead salts in other species.

The value of the acute oral LD50 of lindane in mice found here, 98 mg/kg, with 95% confidence limits of 80 and 120 mg/kg, is quite comparable to the value listed by Negherbon (1959), 86 mg/kg. The same author also states that among higher animals, the acute oral LD50 of lindane has a mean of 125 mg/kg; in the rat, the value is 180 mg/kg, and in the guinea pig it is 115 mg/kg. The mouse, therefore, appears to be somewhat more susceptible to lindane intoxication than other species; thus, mice might be of immense value in assessing the

effects of lindane in mammals. When given acutely or subacutely to experimental animals, lindane elicits signs of central nervous hyperexcitability, including convulsions (Negherbon, 1959). In man, a dose of 45 mg has been reported to produce convulsions, although 11 subjects of a group of 15 noted no effects following this dose (Negherbon, 1959).

This study showed the acute oral LD50 of dieldrin in mice to be 45 mg/kg, with 95% confidence limits of 27 and 75 mg/kg, which include the value of the acute LD50 of dieldrin in rodents (route not specified) given by Dreisbach (1963), 40 mg/kg. Negherbon (1959) gives the acute oral LD50 of dieldrin in the rabbit as 150 mg/kg, and in the dog as 80 mg/kg. The mouse, again, seems to be more sensitive to intoxication by this agent than other species. Acute intoxication is manifest as hyperexcitation of the nervous system, with such signs as tremors and convulsions (Negherbon, 1959). In man, no chronic injury has been noted, but an acute dose of over 10 mg/kg has resulted in symptoms of serious intoxication.

The mice were given the highest dose of lead (150 ppm) consistent with absence of significant diarrhea, in an attempt to attain maximum absorption of lead. At this level of lead intake, the LD50's of lindane and dieldrin would be expected to be changed by lead if a change were possible at all. Measurements indicated a daily water consumption rate of about 10 ml per animal; on this basis, mice consuming water containing 150 ppm lead ingested about 1.5 mg of

lead per day. This rate of lead intake is sufficient to induce severe symptoms of chronic intoxication in man, whose body weight is 3000 times that of a mouse (Kehoe et al., 1940). The lethality studies indicated that the acute LD50's of lindane and dieldrin are no different in the presence of chronic lead intoxication than they are in its absence. Thus, if extrapolation to humans is valid, an individual chronically exposed to lead would be expected to react to an acute exposure to lindane or dieldrin in the same manner as any other person. Those chronically exposed to lead apparently need take no precautions to avoid lindane or dieldrin exposure beyond those any other person would take, if death in response to a single exposure is the only concern. And, if exposure to one of these pesticides were to occur, the prognosis would be no different if the patient were or were not chronically exposed to lead.

It must be kept in mind, however, that lethality is often a difficult pattern to change. It is a gross parameter, and not always a sensitive one. Also, when dealing with humans, death is not the only concern; an individual's health and well-being are also taken into consideration. So, although these studies indicate that chronic lead intoxication does not alter the lethality of either lindane or dieldrin when given acutely, and that an acute preadministration of dieldrin does not change the acute LD50 of lead, the conclusion must not be drawn that these agents are not exerting dangerous toxic effects on the body.

IV. STUDIES ON BIOCHEMICAL EFFECTS AND HEMATOLOGIC FACTORS

Introduction

Elevations in levels of urinary delta-aminolevulinic acid (ALA) are observed in chronic lead poisoning, apparently resulting from interference with the action by lead of ALA dehydrase (Byers, 1959; Albahary, 1964; Chisolm, 1964; Greengard, 1966). This may be a result of binding of lead to the enzyme, or the interference of lead with a cofactor, such as magnesium.

Levels of urinary porphyrins, especially coproporphyrin III, are increased in lead intoxication (Greengard, 1966). The effect is likely due to the blockade of the incorporation of iron into protoporphyrin, thus resulting in the build-up of protoporphyrin and its precursors (Figure 3-1).

Hematologic parameters are of great value in assessing lead poisoning in the clinic (Dreisbach, 1963). Impairment of hemoglobin biosynthesis at apparently two points, conversion of ALA to porphobilinogen, and incorporation of iron into protoporphyrin, results usually in microcytic, hypochromic anemia (Dreisbach, 1963).

The interference of lead with hemoglobin biosynthesis suggests that the metal might interfere with the biosynthesis of other heme proteins. One such heme protein is cytochrome P-450, so called

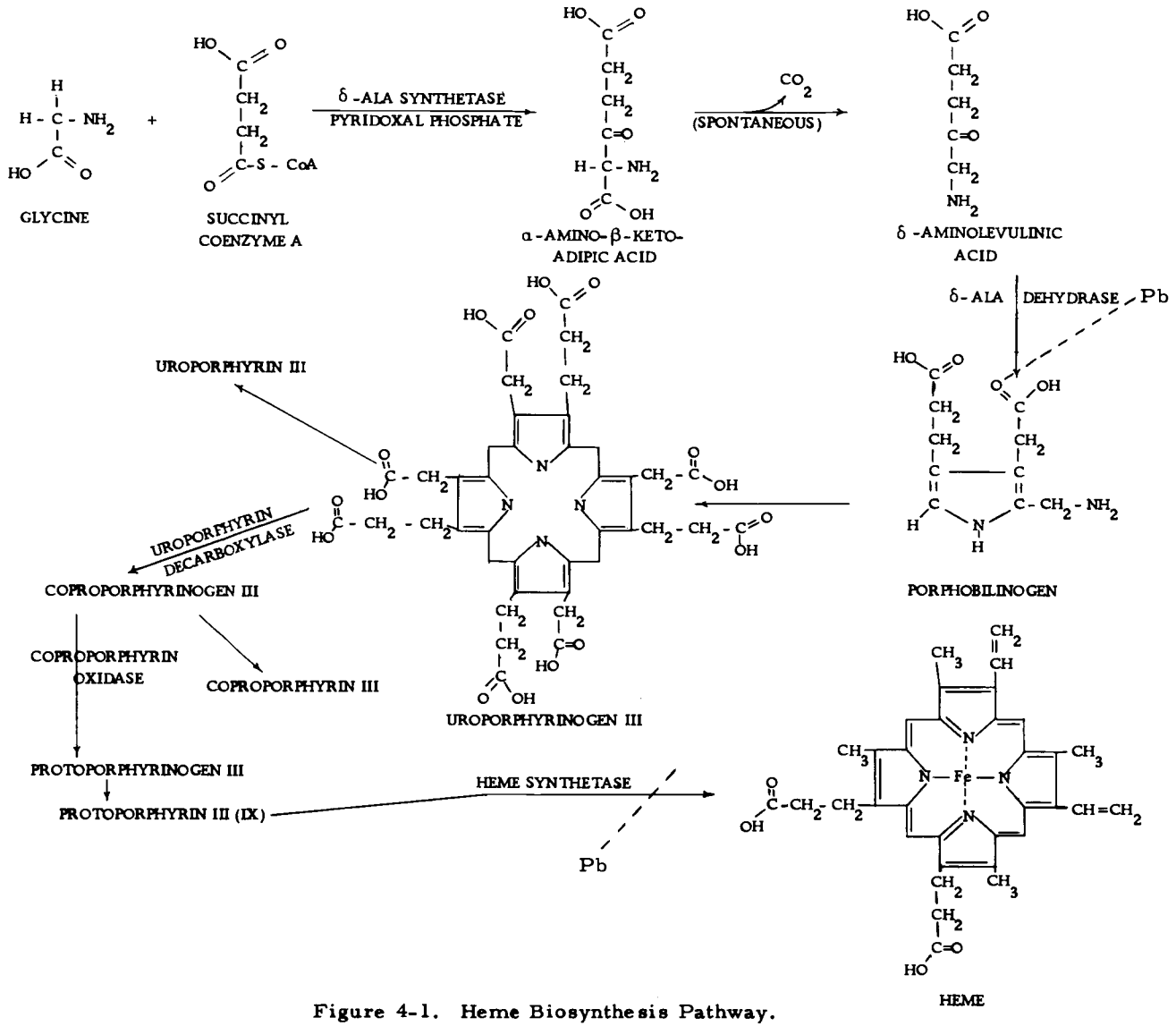

Figure 4-1. Heme Biosynthesis Pathway.

because it is a protein, and its carbon monoxide complex has an optical density maximum at 450 mμ (Garfinkel, 1958; Omura and Sato, 1964). Cytochrome P-450 was first found by Klingenberg (1958), and is an iron-containing compound involved in microsomal oxidation (Estabrook, Cooper and Rosenthal, 1963; Orrenius, Gallner and Ernster, 1964). It appears to be the oxygen activating enzyme for mixed-function oxidases involved in microsomal electron transport (Omura, Sato, Cooper, Rosenthal and Estabrook, 1965). Mixed-function oxidases mediate various metabolic reactions whereby molecular (atmospheric) oxygen reacts with various compounds in the body in TPNH-requiring reactions. Cytochrome P-450 activates the molecular oxygen so that it can react with the compound which is undergoing metabolism. It is the terminal oxidase in this mixed-function oxidase system (Cooper, Levin, Narasimhulu, Rosenthal and Estabrook, 1965).

Ockner et al. (1961) reported that rats fed 0.2% benzene hexachloride in the diet exhibited increases in urinary delta-ALA levels. Since this symptom is also seen in lead intoxication, studies were undertaken to determine the effects of lead, lindane, and combinations of the two agents on urinary ALA.

Gillett and Chan (1969) have shown that lindane is capable of inducing hepatic microsomal drug-metabolizing enzymes. If lindane were to induce those enzymes responsible for the biosynthesis of

heme, perhaps lindane administration would exert some effect on the pathologic alteration in heme biosynthesis elicited by lead. There is evidence that agents possessing the ability to induce hepatic microsomal enzymes possess the ability to induce the synthesis of ALA synthetase (Granick and Urata, 1963; Granick, 1966; Marver, Collins, Tschudy and Rechcigl, 1966; Tschudy, Waxman and Collins, 1967). There would exist some clinical significance for those individuals suffering from various porphyric disorders, including acute intermittent porphyria (AIP). In these disorders, large amounts of porphyrins are excreted in the urine. There is an accompanying central effect which involves bizarre psychic and behavioral changes. Persons afflicted with AIP seem to be quite sensitive to the barbiturates, in that an attack of porphyria can be brought on by administration of certain of the barbiturates, especially those with a high potential for inducing microsomal enzymes. The question before us now is, "What effects do lead and lindane exposure have on urinary porphyrins and on ALA?" The etiology of AIP is unknown; a study of the effects of lead and lindane intoxication on urinary porphyrins may shed some light on this area.

Lindane is reported to depress bone marrow function (West, 1967). This would tend to produce reductions in hemoglobin concentration and numbers of cells in the blood. Lindane's ability to induce hepatic microsomal enzymes, if it is synonymous with ability

to induce ALA synthetase, might tend to counteract the effects of depression of bone marrow function. Thus a balance would have to be attained between depression of bone marrow function and induction of enzymes. When lead is added to this system, the degree of complexity increases due to lead's effect on heme synthesis.

It is the purpose of this series of studies to determine the effects of lead, lindane, and combinations of both agents on various biochemical and hematologic parameters in mice, with the hope of elucidating to some extent the mechanisms of these agents with regard to alterations they might have on heme biosynthesis and cytochrome P-450 biosynthesis. Alterations found may have implications for individuals suffering from porphyric disorders.

Methods

Urinary delta-aminolevulinic acid (ALA) was determined by the method of Davis and Andelman (1967). Urine was collected by placing six mice in a circular wire metabolism cage six and one-half inches in diameter and five and one-half inches in height, the bottom of which was coated with white, non-lead, rust-resistant paint. Urine was collected in a graduated cylinder containing 0.2 ml of 50% acetic acid solution. Collection continued for 24 hours. Water was available to the mice at all times. Urine samples were refrigerated after collection, and analyses performed within four days.

The method of analysis involved a preliminary isolation of ALA by means of two ionic exchange resins (see Figure 4-2). One ml of urine was passed through three grams of an anionic exchange resin, AG 1-X8, 100-200 mesh, acetate form, in the barrel of a disposable glass syringe of 10 milliliters volume. This step served to remove porphobilinogen from the sample. The urine was next passed through three grams of a cationic exchange resin, AG 50W-X4, 100-200 mesh, hydrogen ion form, contained in a syringe barrel as was the anionic resin. The ALA was retained on the cationic column, and all other substances passed on through. Three rinses with eight milliliters of distilled water served to wash any remaining ALA onto the cationic column, and to remove any other substances from that column. The ALA was then eluted from the cationic column with seven milliliters of 1 N sodium acetate. ALA was eluted into a 15-ml centrifuge tube which had been previously marked at the 10-ml level. After elution, 0.2 ml of purified grade pentanedione was added. The mixture was brought up to a total volume of 10 milliliters by means of acetate buffer (pH 4.6). After boiling the sample for ten minutes, and cooling it to room temperature again, two milliliters of the sample were placed in a cuvette, and two milliliters of modified Ehrlich's Reagent were added. After 15 minutes optical density at 553 nm was read, using a Bausch and Lomb "Spectronic 20" colorimeter. After use, columns were cleaned by the method of Wilkinson (1968)

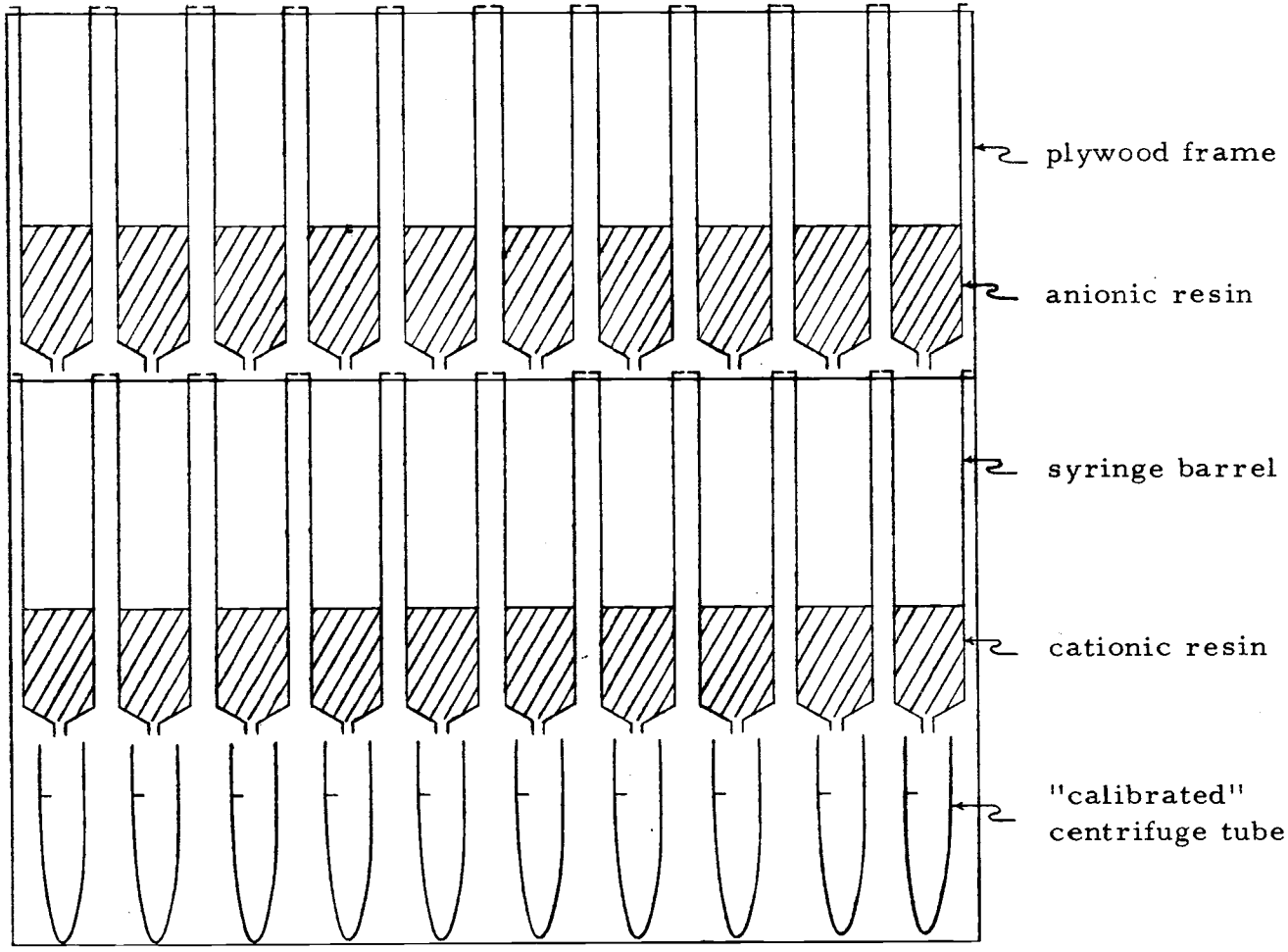

Figure 4-2. Apparatus for isolation of delta-aminolevulinic acid.

for reuse. The anionic columns were rinsed with 1 N sodium acetate until no chloride ion was detectable in the eluate. The cationic columns were cleaned by allowing 2 N sodium hydroxide to sit in them overnight; they were then rinsed successively with distilled water, 4 N hydrochloric acid, 2 N hydrochloric acid, 1 N hydrochloric acid, and distilled water.

Porphyrin analyses were performed after the method of Philips (G. K. Turner Associates' Manual of Fluorometric Clinical Procedures). Urine was collected as for ALA analyses, except that the collecting graduated cylinder contained 0.2 ml of 10% sodium bicarbonate instead of acetic acid. In all analyses, five milliliters of urine were used. The method involved buffering the sample with an equal volume of phosphate buffer (pH 4.8), and adding 10 ml of distilled water and 100 ml of ethyl acetate. Coproporphyrin was extracted into the ethyl acetate layer by shaking the mixture in a separatory funnel. Following separation of the two porphyrins, coproporphyrin was extracted from the ethyl acetate by washing ten times with five ml of 1.5 N hydrochloric acid, and combining washings. The amount of fluorescence at 595 nm which the sample emitted upon exposure to light of 405 nm wavelength was read from a Turner fluorometer. Concentration was determined by comparison to a standard, using the equation:

$$\frac{\text{micrograms of coproporphyrin}}{\text{five ml of urine}} = \frac{\text{reading of sample} \times 2.31}{\text{reading of standard}}$$

The constant in the equation is for the purpose of correcting for the solubility of coproporphyrin in ethyl acetate-saturated 1.5 N hydrochloric acid, and to adjust for a 50-ml extraction volume.

Uroporphyrin was adsorbed onto 0.5 gram of aluminum oxide, which was subsequently washed with 20 ml of half-saturated sodium acetate and 40 ml of distilled water. Extraction was then carried out, using ten portions of five ml of 1.5 N hydrochloric acid. Fluorometric analysis was performed as for coproporphyrin. Concentration was calculated by means of the equation:

$$\frac{\text{micrograms of uroporphyrin}}{\text{five ml of urine}} = \frac{\text{reading of sample x 1.74}}{\text{reading of standard}}$$

The constant in this equation serves the same purposes as the constant in the preceding equation, in addition to compensating for the use of coproporphyrin rather than uroporphyrin as the standard.

Hemoglobin concentration in the blood was determined by conversion of hemoglobin to cyanmethemoglobin (Frankel and Reitman, 1963). Optical density was read on a Bausch and Lomb "Spectronic 20" colorimeter.

Hematocrit was obtained by centrifugation of capillary tubes of blood obtained by cardiac puncture. Tubes were sealed with "Critoseal" and hematocrits were read from a "Spirocrit" microhematocrit reader (obtained from Van Waters and Rogers).

Blood for red cell count was obtained by cutting the tip of the

tail. Erythrocytes were counted visually, using a hemocytometer, according to the method of Berkson, Magath and Hurn (1940).

Mean corpuscular volume is used as an index of erythrocyte size. This index was obtained by multiplying the hematocrit by 10, and dividing this product by the red cell count in millions (Wintrobe, 1961). The result is in cubic microns.

Mean corpuscular hemoglobin is the average amount (mass) of hemoglobin per red cell. This parameter was calculated by dividing the hemoglobin concentration of the blood (in grams per liter) by the red cell count in millions (Wintrobe, 1961). Units are micro-micrograms.

Mean corpuscular hemoglobin concentration is the average concentration of hemoglobin in the red cells, and is an indication of the percent saturation of red cells with hemoglobin. This parameter was obtained by dividing the hemoglobin concentration (in grams per 100 ml of blood) by the hematocrit (Wintrobe, 1961). Units are grams per 100 ml of red cells.

Cytochrome P-450 was determined in mouse liver after the method of Omura et al. (1964). The liver of a freshly-killed mouse was placed in 10 ml of ice-cold 1.15% KCl. It was then homogenized in a ground glass mortar with a Teflon pestel. The homogenate was centrifuged at 9000 x g for ten minutes at 4°C. The supernatant was then centrifuged at 100,000 x g for 90 minutes at 4°C; the resulting

pellet was rinsed with ice-cold 1.15% KCl, and resuspended in the same solution, after which it was recentrifuged at 100,000 x g for 90 minutes at 4°C. The pellet was resuspended in ice-cold 1.15% KCl; of this, 0.5 ml was diluted with ice-cold buffer (pH 7.0) to a volume of four ml. From this sample, 0.1 ml was used to determine protein concentration by the method of Lowry, Rosebrough, Farr and Randall (1951), which involved a Folin-Ciocalteu phenol reagent. The determination of cytochrome P-450 was carried out as follows: to the microsomal suspension was added a few milligrams of sodium dithionite to reduce the microsomes; the suspension was then divided and placed in two cuvettes; carbon monoxide gas, generated by mixing concentrated formic acid and concentrated sulfuric acid, was bubbled through the suspension in one cuvette for one minute; the optical density differences between the two suspensions were then determined at 450 nm and 500 nm, using a double-beam Bausch and Lomb "Spectronic 600" spectrometer. The optical density difference at 500 nm was subtracted from the optical density difference at 450 nm; the resulting difference was divided by the extinction coefficient of cytochrome P-450, 91 mM^{-1} cm^{-1}, and by the concentration of total protein in the suspension (Omura and Sato, 1963). Results are therefore expressed as nanomoles of cytochrome P-450 per milligram of protein.

Results

Administration of lead increased levels of urinary ALA excretion in a dose-related fashion, as is seen in Table 4-1. At the highest level of lead given, urinary concentration of ALA was 11 times that in controls. Lindane did not significantly alter levels of urinary excretion of ALA in mice in this study. This contrasts with the results found by Ockner et al. (1961) in their studies in the rat.

Table 4-2 shows the effects of lead and lindane treatment on urinary coproporphyrin. Although none of the levels was significantly different from the control level when mice were treated with lead only, a tendency to increase urinary coproporphyrin is evident. All values of lindane-treated animals are seen to have exceeded their respective non-lindane-treated counterparts, except in one instance. With both agents working to raise urinary coproporphyrin levels, a significant increase was finally attained when lead at the 300 ppm level was administered concomitantly with lindane. This could be explained by a blockade of heme synthesis by lead at the point of incorporation of iron into protoporphyrin in conjunction with acceleration of heme synthesis by induction of heme-synthesizing enzymes by lindane.

It is apparent that neither agent exerted an appreciable effect on uroporphyrin excretion (Table 4-3).

The interference of lead with heme synthesis should lead to a depression of hemoglobin concentration in the blood; this is what was

Table 4-1. Urinary concentrations of delta-aminolevulinic acid in CF #1 mice following administration of lead for eight weeks and of lindane for two weeks.

Level of lead given	Dose of lindane given	
	0	40 mg/kg
	Mean mg ALA per 100 ml urine ± S. E.	
0	0.45 ± 0.13 (9)[a]	0.59 ± 0.19 (9)
75 ppm	0.83 ± 0.96* (4)	1.95 ± 0.92* (5)
150 ppm	3.52 ± 2.05* (4)	2.18 ± 1.11* (5)
300 ppm	5.02 ± 2.62* (7)	4.53 ± 1.82* (7)

[a]Number of analyses (each replicated three times).

*Mean significantly different from mean of control group, which received neither lead nor lindane ($P < .05$).

Table 4-2. Urinary concentrations of coproporphyrin III in CF #1 mice following administration of lead for eight weeks and of lindane for two weeks.

Level of lead given	Dose of lindane given	
	0	40 mg/kg
	Mean micrograms per ml ± S. E.	
0	0.214 ± 0.026 (10)[a]	0.258 ± 0.062 (9)
75 ppm	0.257 ± 0.029 (6)	0.209 ± 0.026 (6)
150 ppm	0.226 ± 0.030 (7)	0.270 ± 0.087 (6)
300 ppm	0.267 ± 0.010 (7)	0.373 ± 0.101* (6)

[a]Number of analyses.

*Mean significantly different from control mean, as in Table 4-1 ($P < .05$).

Table 4-3. Urinary concentrations of uroporphyrin III in CF #1 mice following administration of lead for eight weeks and of lindane for two weeks.

Level of lead given	Dose of lindane given	
	0	40 mg/kg
	Mean micrograms per ml ± S. E.	
0	0.012 ± 0.002 (6)[a]	0.008 ± 0.005 (5)
75 ppm	0.016 ± 0.004 (4)	0.014 ± 0.003 (4)
150 ppm	0.012 ± 0.002 (5)	0.013 ± 0.002 (4)
300 ppm	0.012 ± 0.0005 (5)	0.014 ± 0.005 (4)

[a]Number of analyses

found when lead was administered in concentrations over 75 ppm (Table 4-4 and Figure 4-3). The effect seemed to be dose-related; the change was not great enough to produce a statistically significant difference until lead was given at 300 ppm. Lindane appeared to have no dramatic effect on hemoglobin concentration when given by itself. However, an interesting effect was seen in that lindane administration appeared to prevent the fall in hemoglobin concentration caused by administration of lead. Thus, when hemoglobin concentration was depressed by the two higher levels of lead given, the difference in hemoglobin concentrations between groups which received lindane and groups which did not receive lindane was greater in the case of lead-treated animals than in the case of animals which did not receive

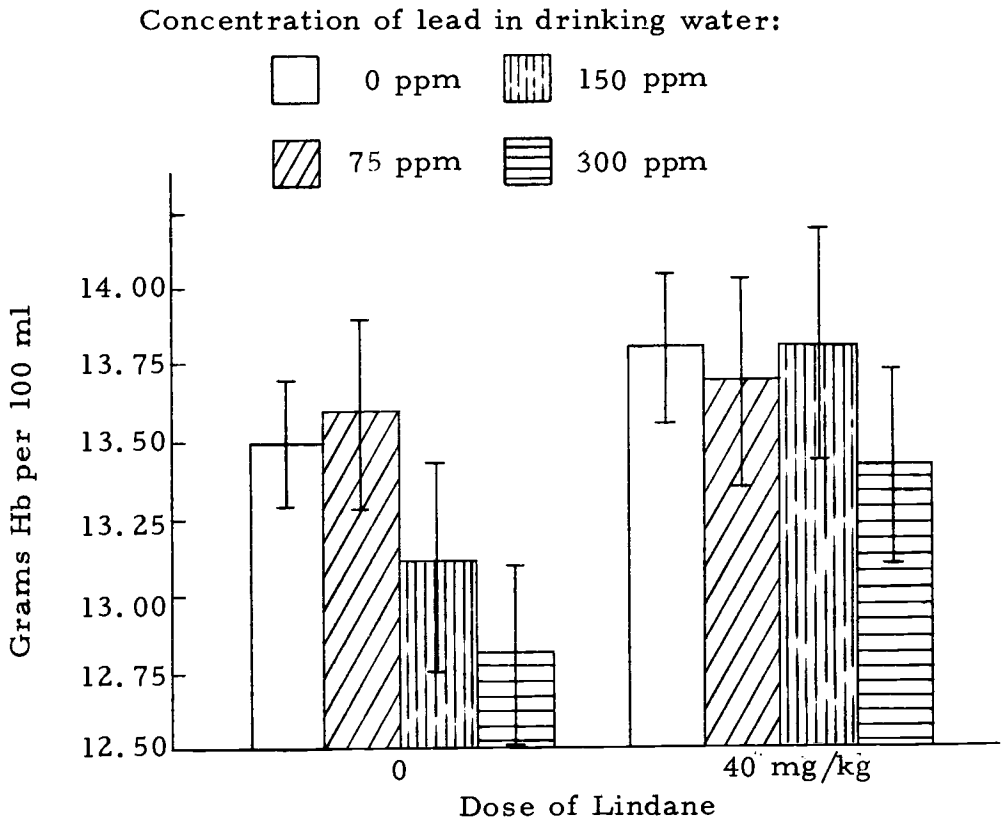

Figure 4-3. Hemoglobin (Hb) concentration in blood of CF #1 mice following administration of lead for eight weeks and of lindane for two weeks. Bars are means; vertical lines are standard errors.

Table 4-4. Hemoglobin concentrations in the blood of CF #1 mice following administration of lead for eight weeks and of lindane for two weeks.

Level of lead given	Dose of lindane given	
	0	40 mg/kg
	Mean grams per 100 ml ± S. E.	
0	13.5 ± 0.22 (85)[a]	13.8 ± 0.25 (88)
75 ppm	13.6 ± 0.31 (34)	13.7 ± 0.34 (32)
150 ppm	13.1 ± 0.34 (35)	13.8 ± 0.36 (34)
300 ppm	12.8 ± 0.30* (49)	13.4 ± 0.31 (47)

[a]Number of animals.
*Mean significantly different from control mean, as in Table 4-1 ($P < .05$).

lead. Thus, lindane appears to have been capable of accelerating heme biosynthesis until a limiting concentration of hemoglobin was attained in the blood. When hemoglobin concentration was not depressed, as in mice which did not receive lead, no effect of lindane on hemoglobin was seen; but when lead depressed the synthesis of heme, and therefore the concentration of hemoglobin in the blood, lindane brought about a full compensation of the depression, but was unable to raise hemoglobin concentrations above the control level.

Lead administration elicited a dose-related fall in hematocrit, both in the presence and in the absence of lindane administration (Table 4-5 and Figure 4-4). The decrease was not statistically

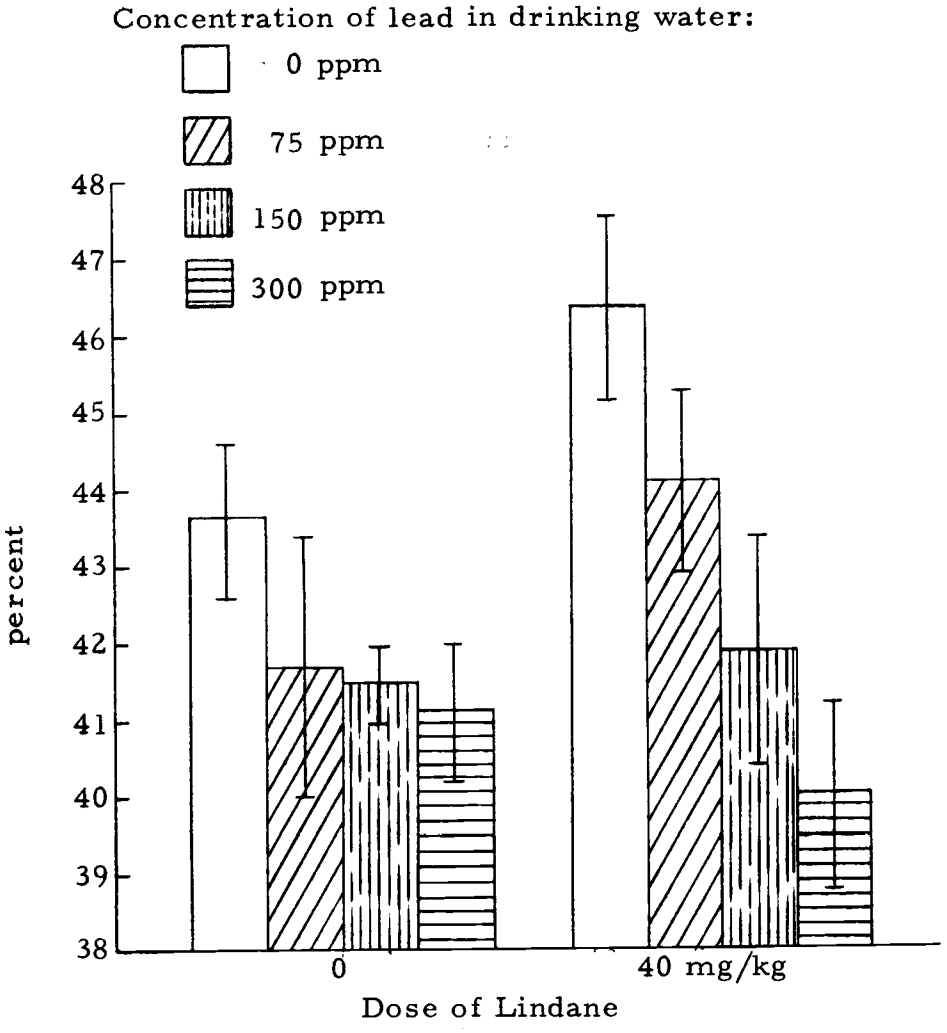

Figure 4-4. Hematocrits of CF #1 mice following administration of lead for eight weeks and of lindane for two weeks. Bars are means; vertical lines are standard errors.

Table 4-5. Hematocrits of CF #1 mice following administration of lead for eight weeks and of lindane for two weeks.

Level of lead given	Dose of lindane given	
	0	40 mg/kg
	Mean ± S. E.	
0	43.7 ± 1.0 (34)[a]	46.4 ± 1.2* (35)
75 ppm	41.7 ± 1.7 (16)	44.1 ± 1.2 (16)
150 ppm	41.5 ± 0.5 (18)	41.9 ± 1.5* (18)
300 ppm	41.1 ± 0.9* (16)	40.0 ± 1.2* (16)

[a]Number of animals.
*Mean significantly different from control mean, as in Table 4-1 ($P < .05$).

significant at the 95% level until the highest concentration of lead (300 ppm) was given. Lindane, when given alone, produced a rise in hematocrit. This apparent polycythemia may be a relative one, in that plasma volume may have decreased, rather than the number of red cells having increased. When lead was given to lindane-treated mice, a dose-related fall in hematocrit was elicited as in the mice which had not received lindane; but when lindane was present, the effect of lead was more pronounced; i. e., the differences between any two groups were larger in lindane-treated animals than in those not given lindane. It was almost as if lindane treatment served to accentuate the effect of lead intoxication on this parameter.

Lead tended to reduce erythrocyte count, although not to a statistically significant degree; indeed, in some cases the means for lead-treated mice (75 ppm or 300 ppm lead) were found to exceed the mean for controls with regard to this parameter. This was consistently the case with 150 ppm lead (Figure 4-5). Lindane usually increased red cell count slightly over the respective means of groups not receiving lindane, although again, the increase was not statistically significant, and in some experiments lindane lowered red cell count irrespective of the level of lead given.

It may well be more difficult to depress red cell levels in mice than in other species, especially man. This is indicated by the increased regenerative capacity of erythrocytes in rodents, especially smaller forms, as compared to man (Altman and Dittmer, 1964). Another difficulty with this parameter as a test in assessing intoxication is the inherent variability of red cell counts. Thus, standard errors are maintained at relatively high levels.

Although none of the means for the mice receiving lead only is significantly different from control means, the tendency for mean corpuscular volume to fall is apparent (Table 4-6). Lindane had little effect on this parameter, but when lindane was given with 300 ppm lead, a significant fall was seen. Thus, although neither lead nor lindane was effective in producing a significant depression in mean corpuscular volume, when lindane was given with the highest level of

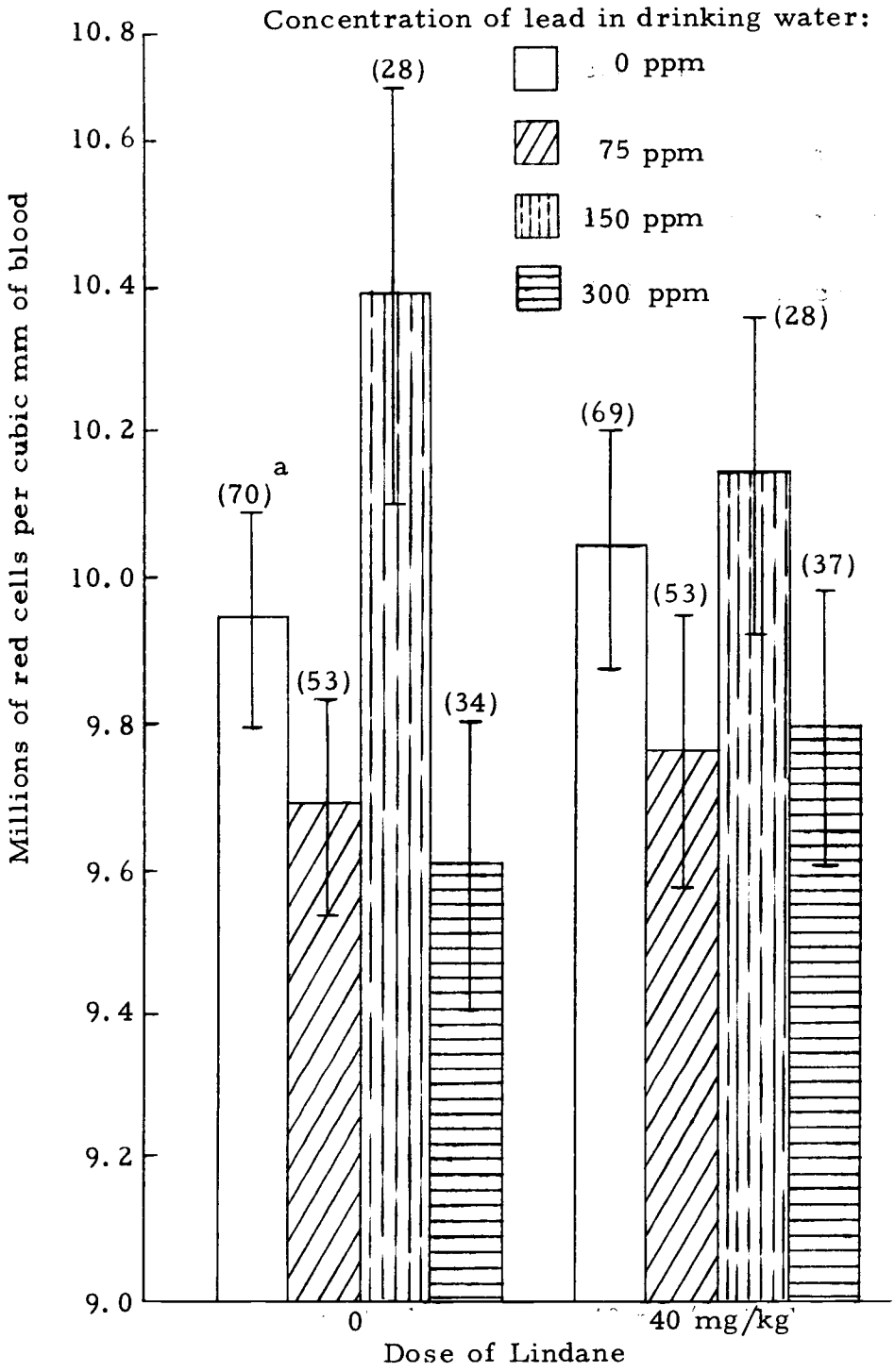

Figure 4-5. Red cell counts in CF #1 mice following administration of lead for eight weeks and of lindane for two weeks. Bars are means; vertical lines are standard errors.

[a]Number of animals.

Table 4-6. Mean corpuscular volumes of CF #1 mice following administration of lead for eight weeks and of lindane for two weeks.

Level of lead given	Dose of lindane given	
	0	40 mg/kg
	Mean cubic microns ± S. E.	
0	44.4 ± 1.0 (69)[a]	45.5 ± 1.4 (67)
75 ppm	44.2 ± 1.2 (49)	45.9 ± 1.0 (49)
150 ppm	43.4 ± 1.2 (16)	42.5 ± 1.6 (16)
300 ppm	41.8 ± 1.1 (32)	41.7 ± 1.3* (33)

[a]Number of animals.
*Mean significantly different from control mean, as in Table 4-1 ($P < .05$).

lead, a decrease which was significant at the 95% level of confidence was seen.

Lead showed a tendency to lower mean corpuscular hemoglobin (Table 4-7). This parallels the results of the study of hemoglobin concentration in the blood. Lindane increased mean corpuscular hemoglobin when the two higher levels of lead acetate were given, a result not unexpected from the increases observed in hemoglobin concentration due to lindane at these levels of lead administration.

Table 4-8 shows that lead brought about a rise in mean corpuscular hemoglobin concentration at all levels of administration. Lindane caused a slight decrease in most instances. The effects of

Table 4-7. Mean corpuscular hemoglobin in CF #1 mice following administration of lead for eight weeks and of lindane for two weeks.

Level of lead given	Dose of lindane given	
	0	40 mg/kg
	Mean micro-micrograms ± S. E.	
0	13.6 ± 0.3 (85)[a]	13.9 ± 0.4 (83)
75 ppm	13.9 ± 0.4 (33)	14.0 ± 0.4 (32)
150 ppm	12.7 ± 0.3* (48)	13.6 ± 0.3** (50)
300 ppm	12.7 ± 0.5* (33)	14.4 ± 0.7** (30)

[a]Number of animals.

*Mean significantly different from control mean, as in Table 4-1 ($P < .05$).

**Mean significantly different from group receiving the same level of lead but not lindane ($P < .05$).

Table 4-8. Mean corpuscular hemoglobin concentration in CF #1 mice following administration of lead for eight weeks and of lindane for two weeks.

Level of lead given	Dose of lindane given	
	0	40 mg/kg
	Mean grams per 100 ml ± S. E.	
0	30.4 ± 0.3 (65)[a]	30.9 ± 0.4 (68)
75 ppm	32.6 ± 0.7* (33)	31.0 ± 0.6 (32)
150 ppm	32.0 ± 0.8* (34)	31.1 ± 0.4 (34)
300 ppm	32.8 ± 1.0* (16)	32.2 ± 0.9* (16)

[a]Number of animals.

*Mean significantly different from control mean, as in Table 4-1 ($P < .05$).

lead administration are seen in lindane-treated mice as well as in those not given lindane. The highest level of lead was capable of overcoming the effect of lindane on this parameter.

The average values of the preceding hematologic parameters, as given by Altman et al. (1964) are (mean and range): hemoglobin, 14.8 (10-19) grams per 100 ml of blood; hematocrit, 41.5% (no range given); red cell count, 9.3 million (7.7-12.5 million); mean corpuscular volume, 49 (48-51) cubic microns; mean corpuscular hemoglobin, 16 (15.5-16.5) micro-micrograms; mean corpuscular hemoglobin concentration, 36 (33-39) grams per 100 ml of red cells.

Lead, when given alone, reduced levels of cytochrome P-450 in the liver (Table 4-9). The effect appeared to be dose-related; the highest level of lead depressed levels of this heme protein by nearly 50%. Even the lowest level of lead given reduced it to a statistically significant degree. Lindane raised cytochrome P-450 levels regardless of the dose of lead given, and the increase, which averaged 83%, was statistically significant in three of the four instances of lindane administration.

Discussion

In general, the studies on hematologic and biochemical parameters agree with the concept that hemoglobin biosynthesis is impaired by lead. That lead blocks the biosynthetic pathway of

Table 4-9. Levels of cytochrome P-450 in livers of CF #1 mice following administration of lead for eight weeks and of lindane for two weeks.

Level of lead given	Dose of lindane given	
	0	40 mg/kg
	Mean nonamoles per mg protein ± S. E.	
0	1.18 ± 0.08 (15)[a]	1.48 ± 0.13** (15)
75 ppm	0.80 ± 0.09* (10)	1.70 ± 0.12* ** (15)
150 ppm	1.03 ± 0.14 (10)	1.28 ± 0.21 (10)
300 ppm	0.65 ± 0.05* (10)	1.75 ± 0.15* ** (10)

[a]Number of analyses.

*Mean significantly different from control mean, as in Table 4-1 ($P < .05$).

**Mean significantly different from mean of group receiving the same level of lead but not lindane ($P < .05$).

hemoglobin production is seen in depression of blood hemoglobin concentration, increased urinary ALA levels and increased urinary coproporphyrin levels. This interference with hemoglobin biosynthesis is likely the cause of the decrease in red cell size, mean red cell hemoglobin, and mean red cell hemoglobin concentration. In view of all these changes seen in mice, it is apparent that the mouse is a useful animal in the laboratory investigation of lead toxicity. The reason mice are rarely used in such studies is difficult to surmise. The only parameter which seemed difficult to alter was red cell count; but clinically, this parameter is not consistently affected.

Lead administration was seen to have brought about an elevation in levels of urinary excretion of ALA in a dose-related fashion. These results can be explained by the blockade of ALA metabolism by interference by lead with the enzyme ALA dehydrase, and perhaps also by blockade of the incorporation of iron into protoporphyrin (Greengard, 1966). Such blockade would result in the build-up of precursors, such as ALA.

Lindane was apparently inactivated quite rapidly in the animals used in these studies. If significant amounts had not been inactivated quickly, the LD50 should have been attained after a few days, since one-fourth of the LD50 was given daily. But the failure of mice to die at the rates that might be expected indicates that detoxification was rapid.

No increase in urinary excretion of ALA was observed when lindane was given by itself in this investigation. Lindane is one of the many compounds capable of inducing hepatic microsomal drug-metabolizing enzyme systems (Gillett et al., 1969). Drugs which have this property also seem to be capable of inducing the enzyme ALA synthetase (Granick et al., 1963; Granick, 1966; Marver et al., 1966; Tschudy et al., 1967). If the activity of this enzyme is induced to a greater degree than the activities of those enzymes responsible for the conversion of ALA to prophyrins, an increase in urinary levels of ALA would be expected. However, if both enzyme systems, i.e., those responsible for the biosynthesis of ALA and those responsible for the metabolism of ALA, are induced to about the same extent, no increase in urinary ALA levels would be expected. This appears to have been the situation in this study. The failure to see increases in urinary ALA indicates that one of two possible phenomena has transpired: (1) ALA synthetase was not induced, or (2) ALA metabolizing enzymes were induced to the same extent as ALA synthetase. If the first possibility is true, there should be no further evidence of enzyme induction as we proceed along the pathway of heme biosynthesis. If the second possibility is the true one, we would expect to observe increases in levels of some other heme precursor, or in heme itself, or even in hemoglobin.

At least three factors lead to the conclusion that the second

suggestion is the more plausible one. Levels of hepatic cytochrome P-450 were increased by lindane administration; concentrations of hemoglobin in the blood, although not significantly different from respective controls which had not received lindane, are seen to be consistently greater than concentrations of hemoglobin in the blood of mice which had not received the pesticide; and levels of urinary excretion of coproporphyrin were elevated by lindane administration. Thus, the enzymes bringing about the production and those bringing about the metabolism of ALA may have been induced to about the same extent, so that more ALA was probably formed, and passed on to synthesize coproporphyrin. When lead blocked heme synthesis at the point of incorporation of iron into protoporphyrin, a magnification of the increase in coproporphyrin excretion was seen. This was probably because of the acceleration of coproporphyrin synthesis by lindane coupled with the blockade of the metabolism of coproporphyrin by lead. The enzymes responsible for the production of uroporphyrin III from uroporphyrinogen III were apparently not induced by lindane, while those involved in the synthesis of coproporphyrin III from coproporphyrinogen III were induced. Also, the possibility exists that all the enzymes along the "main stream" of the heme-synthesis pathway were induced by lindane, until coproporphyrinogen III was reached; the enzyme which converted coproporphyrinogen III to protoporphyrin was not induced, at least not to the same extent as the enzymes

involved in the biosynthesis of coproporphyrinogen III.

Levels of both of the heme proteins investigated here, namely, hemoglobin and cytochrome P-450, were depressed by lead. It is apparent from this that lead was capable of blocking heme synthesis throughout the body, so that whether the synthesis took place in the bone marrow, as in the case of hemoglobin, or in the liver, as in the case of cytochrome P-450, the biosynthetic pathway was at least partially blocked. Since lead interferes with hemoglobin synthesis at at least two points, it is not surprising that red cell count would be depressed by lead. As mentioned previously, this symptom is sometimes observed in humans poisoned with lead. And, as was found in this study, red cell count may also be increased or left unchanged by lead intoxication.

Lindane, being an inducer of enzymes, might be expected to bring about an increase in hemoglobin biosynthesis, especially since those agents capable of inducing hepatic microsomal drug-metabolizing enzymes also possess the ability to induce ALA synthetase activity (Granick et al., 1963; Granick, 1966; Marver et al., 1966; Tschudy et al., 1967; Gillett et al., 1969). Indeed, lindane prevented the fall in hemoglobin concentration produced by lead. The induction of the enzyme activity of the heme-synthesis pathway might be expected to result in increased red cell production; however, Dreisbach (1963) and West (1967) reported a depressant effect of lindane on bone marrow

function, manifest as aplastic anemia. This depressant effect could be expected to counteract any increase in hemoglobin biosynthesis. Thus, a balance may exist between enzyme induction and bone marrow function depression.

Another factor complicating the situation is fluid balance, which may or may not have been altered by the relatively high doses of lindane employed in this study. If plasma volume were decreased by lindane, an apparent increase in hemoglobin concentration would be observed. Likewise, red cell count and hematocrit would appear to increase; this effect would be due not to an actual increase in the "solute," but to a decrease in the "solvent," resulting in a relative, rather than an absolute, increase in "solute." This possibility remains to be explored.

Although lindane by itself produced no measurable effect on hemoglobin concentration, it was capable of overcoming the effects of lead in the face of reduced hematocrit. The slight increases in hemoglobin levels seen when higher doses of lead were given together with lindane could possibly be explained by an induction of the enzymes which were inhibited by lead when it was given alone. Since hemoglobin levels in lindane-treated animals never exceeded control levels, despite lindane's ability to induce hemoglobin production in lead-treated animals, it would seem as though the enzyme systems involved in hemoglobin synthesis were operating near a maximum rate

in control animals. Only when the rate of enzyme actions was depressed by lead could it be accelerated by some other stimulus, such as lindane. Although lead depressed hemoglobin production, it did not simultaneously depress the maximum level at which hemoglobin levels in the blood could be maintained. An enzyme inducer could, therefore, stimulate heme synthesis over a wider range in a lead-poisoned animal than it could in a control animal. Lindane's effect here was maximal, i. e., hemoglobin levels were brought back up to what appears to be the ceiling value in every case of lindane administration. Thus, the blockade of heme synthesis by lead was of such a nature that lindane was capable of overcoming it entirely; that is, enzyme interference (by lead) was completely counteracted by enzyme induction (by lindane).

The increase in urinary coproporphyrin upon lindane administration could also be explained on the basis of the induction of biosynthesizing enzymes. The systems involved in the production of this substance seemed to be working at a submaximal rate, and could, therefore, be accelerated above the control level by lindane.

Levels of hepatic cytochrome P-450 were depressed by lead administration (300 ppm) to about 50% of the control level. Thus, lead may be added to the growing list of substances that have been observed to depress levels of hepatic cytochrome P-450. Thyroxin and 3-amino-1, 2, 3-triazole have been shown to lower hepatic cytochrome P-450

levels in rats by about 50% (Kato and Takahashi, 1968; Baron and Tephly, 1969). Gillette, Kamm and Sasame (1968) have demonstrated a depression of hepatic cytochrome P-450 levels in mice by as much as 70% by oral administration of carbon tetrachloride. The depression of levels of hepatic microsomal cytochrome P-450 in this study indicates that certain oxidative metabolic processes, that is, those mediated by that cytochrome, may have been impaired by lead. Some of these processes are those involved in the metabolism of drugs such as barbiturates. Thus, if barbiturate metabolism were impaired, a drug in this class could exert its effects for a longer time than would otherwise by the case. An example is phenobarbital, which Kato (1960a) has shown to possess the ability to induce hepatic microsomal drug-metabolizing enzymes. If it also induced coproporphyrin-synthesizing enzymes, it could exert this effect for a prolonged period of time, and to a greater degree than in the non-lead-poisoned liver because of the interference with phenobarbital-metabolizing enzymes by lead. Thus, phenobarbital would elicit symptoms of porphyria, including increases in coproporphyrin excretion in the urine. In individuals afflicted already with the disease acute intermittent porphyria, this added porphyric effect could lead to serious consequences because of lead's interference with phenobarbital-metabolizing enzymes.

The central effects of the phenobarbital would persist for a

longer period of time also; perhaps the central stimulatory effect of lead could at least partially counteract the depression brought about by the phenobarbital.

The same argument could hold for any number of drugs or other potentially toxic agents that depend on cytochrome P-450 for their metabolism; i. e., metabolism might be impaired, and the drug would exert its effect for a longer time, and perhaps more severely. It should be noted here that Holtzman, Gram, Gigon and Gillette (1968) suggest that cytochrome P-450 reductase activity may be of more importance than concentrations of the cytochrome in the liver.

Lindane is a compound that may depend on cytochrome P-450 for metabolism. If lead impaired the ability of the liver to metabolize lindane, it could exert a greater effect in the presence of lead poisoning. This could have been the case with regard to hemoglobin levels in the blood. If the metabolism of lindane were depressed by lead's effect on hepatic cytochrome P-450, more lindane would be available for induction of enzymes. Thus, lindane could have exerted a greater effect on hemoglobin synthesis in the bone marrow when lindane metabolism was depressed by lead. It was seen, indeed, that lindane produced a greater increase in hemoglobin levels when liver function was compromised by lead.

Two possibilities are indicated here, neither of which seems to have been explored as yet: either the heme-synthesizing enzymes in

the bone marrow are induced (no information seems to be available in the literature with regard to induction of bone marrow enzyme systems); or, if this is not the case, there would appear to be only one other source of increased amounts of ALA synthetase, and therefore only one non-marrow source of the enzyme, that source being the organ in which enzyme systems are known to be induced, namely, the liver.

The increase in levels of hepatic microsomal cytochrome P-450 when lindane was given could probably be explained by induction of the enzymes involved in the biosynthesis of the cytochrome. This may have been a result of induction of ALA synthetase activity, or of the activities of several other eyzymes along the biosynthetic pathway of heme. Phenobarbital is an inducer of enzymes, which is capable of eliciting increases in levels of hepatic cytochrome P-450. Following from three to five daily intraperitoneal injections of from 40 to 100 mg/kg of phenobarbital in rats, increases in levels of hepatic cytochrome P-450 of from 240% to 300% have been reported (Orrenius and Ernster, 1964; George and Tephly, 1968; Baron et al., 1969; Guarino, Gram, Gigon, Greene and Gillette, 1969; Sladek and Mannering, 1969). Thus, the 83% increase found in this study appears to indicate that lindane is not as efficient an enzyme inducer in mice as is phenobarbital in rats. The author is not aware of any other reports of attempts to induce production of hepatic microsomal cytochrome P-450 in mice by phenobarbital or by any other agent. It is possible that

induction of cytochrome P-450, and of drug-metabolizing enzymes, is more difficult in mice than in rats. This seems to be the case with regard to enzyme induction by DDT (Hart and Fouts, 1965; Conney, 1967).

In the human, lead intoxication usually results in a microcytic, hypochromic anemia (Dreisbach, 1963). In these studies with the mouse, a microcytic anemia was observed, but it tended to be of the hyperchromic type. Although hemoglobin biosynthesis was interferred with by lead, the size of the red cells was decreased proportionately more, so that the mean erythrocyte hemoglobin concentration rose.

Lindane exerted no effect on red cell size, and increased mean corpuscular hemoglobin only when hemoglobin production was depressed by the two higher levels of lead. Lindane apparently did not alter mean corpuscular hemoglobin concentration.

In general, the effects of combinations of lead acetate intoxication and lindane intoxication on hematologic and biochemical parameters appear to consist of simple algebraic sums of the effects of intoxication with each agent alone; the exception is in hemoglobin concentrations. No supra-additive effects were observed. The additive effect found with coproporphyrin indicates that, when the heme synthesis rate is compromised by one agent, it is less able to withstand an onslaught by the other agent. This would indicate, in contrast to those conclusions drawn from studies of acute lethalities,

that, if this information can be extrapolated to humans, an individual chronically poisoned by lead (whether the intoxication has reached the clinical stage or not) must indeed take special precautions to avoid exposure to lindane. And patients intoxicated with lindane must be protected to a special degree from exposure to lead.

This additive effect may have another result in the clinic. If urinary coproporphyrin is seen to be elevated, a diagnosis of lead poisoning might be made. This could well be true, but there is also the possibility that lead exposure will be overestimated because of the presence of lindane intoxication along with slight to moderate lead poisoning. Thus, the lindane intoxication might not be noticed, and only the lead intoxication would be treated, and that to a greater degree than necessary.

Another consideration is the fact that the results of some of the diagnostic tests may be influenced in opposite directions by lead and lindane. For instance, urinary ALA levels were sometimes depressed by lindane. Thus, the degree of intoxication may be underestimated, and inadequate treatment might result.

The joint actions of lead and lindane at the level of the bone marrow require further study, perhaps in other species. Much valuable information could also be gained by studying the levels of the various enzymes of the heme-biosynthesis system under the influence of lead and lindane administration. Another investigation that is

indicated by the foregoing is a study of changes in fluid balance in lindane intoxication.

V. STUDIES ON THE CENTRAL NERVOUS SYSTEM

Introduction

Lead exerts perhaps its most serious effect, at least in humans, on the central nervous system. In chronic lead poisoning, a condition known as lead encephalopathy may be found. This disorder is characterized by clumsiness, ataxia, headache, insomnia, restlessness, and irritability, progressing to delirium and grand mal-like convulsions, coma, and death. Such symptoms are also characteristic of increased intracranial pressure. Autopsy reveals proliferative meningitis and intense cerebral edema, and at times also punctate hemorrhages, gliosis, and areas of focal necrosis (Harvey, 1965).

The effects of lindane and of dieldrin on the central nervous system are manifest as hyperexcitation. Symptoms include nervousness, tremors, ataxia, tonic-clonic convulsions, and death due to respiratory paralysis (Dreisbach, 1963; Hayes, 1965).

Since lead, lindane and dieldrin are capable of producing neurologic effects, a battery of some of the commonly-used tests of central nervous function was employed to determine the effects of these three agents on the central nervous system of the mouse.

A common test of the effects of drugs on central nervous activity is the measurement of their effects on barbiturate sleep time. Drugs that depress central activity prolong barbiturate sleep time,

whereas central stimulants shorten it. However, another mechanism of shortening barbiturate sleep time is the induction of those enzymes involved in the metabolism of barbiturates (Conney, Michaelson and Burns, 1961). Therefore, shortening of sleep time indicates either increased central activity or induction of hepatic microsomal drug-metabolizing enzymes, or both. Pentobarbital was used here, rather than some other barbiturate such as hexobarbital or phenobarbital, because recovery from sedation depends mainly upon redistribution, although it can be altered by induction of hepatic microsomal drug-metabolizing enzymes.

Pentylenetetrazol (Metrazol) is a central stimulant drug used widely in seizure-susceptibility tests. Administration of many drugs alters the susceptibility of an animal to pentylenetetrazol-induced seizures (Goodman, Toman and Swinyard, 1949). An increase in susceptibility to chemically-induced seizures is termed a decrease of seizure threshold. The effects of lead, lindane and dieldrin administration on this threshold were measured.

Toman (1951) proposed the use of low-frequency electroshock (lfES) in screening drugs for use against psychomotor seizures. When an electric shock is given with frequencies of a few cycles per second, the seizures elicited resemble those seen in psychomotor epilepsy. The technic has also been adapted to determine seizure susceptibility. Since it seemed desirable to determine the effects of lead and lindane

administration on some type of electrically-induced seizures, and since another commonly-used electroshock seizure test, the maximal electroshock seizure test, often results in death when administered to mice, susceptibilities to seizures induced to low-frequency electroshock were measured.

Ataxia and muscular weakness in mice can be estimated by placing the animals on a rotating rod and measuring the length of time they can remain on the rod (Dunham and Miya, 1957). This test is intended to overcome difficulties which may be encountered in other tests of incoordination, such as inexperience on the part of the technician, inability to detect very slight evidences of neurotoxicity, and appearance of a false positive test. It is easier to employ than various tests which measure righting, positional sense, gait and stance, muscle tone, and equilibrium, and was proposed to possess potential value in the testing of skeletal muscle relaxants, convulsants, central depressants, etc. The apparatus was given the name "rotarod" by Plotnikoff, Reinke and Fitzloff (1962).

Methods

The pentobarbital sleep time test measures the length of time an animal is unable to right itself after administration of the drug being tested and pentobarbital. The mice were given pentobarbital sodium, 40 mg/kg, by intravenous injection. This route of

administration was chosen rather than the more traditional intraperitoneal because the loss of the righting reflex occurs much more quickly, thus giving a sharper time of onset. An animal was considered to have regained its righting reflex when it could right itself three times within 30 seconds.

Pentylenetetrazol seizure threshold (PST) was determined by a timed intravenous infusion technic (Fingl and McQuarrie, 1960). A 0.5% (w/v) solution of pentylenetetrazol in saline was infused into a tail vein at a constant rate of 0.4 ml per minute until the mouse exhibited persistent clonus for three seconds. A timer was started when pentylenetetrazol solution first entered the vein, and was stopped when persistent clonic seizures obtained (in the mouse, clonus precedes tonus in pentylenetetrazol seizures). Thus, a seizure threshold was determined for each animal tested. Seizure threshold was expressed as the length of time (in seconds) required to elicit persistent clonic seizures.

Susceptibility to seizures induced by low-frequency electroshock was determined by delivering electroshock through corneal electrodes, after the method of Roman (1951), as modified by Brown, Schiffman, Swinyard and Goodman (1953). Pulses of 0.2 millisecond duration were administered at a frequency of six per second for three seconds by means of a Grass model S4 stimulator. From each drug-treated group of mice to be tested, smaller groups were given electroshock at

different voltages. By plotting the percent of mice in each smaller group giving seizures against voltage on log-probability coordinates, a median effective voltage (EV50; i. e., the voltage sufficient to induce minimal seizures in 50% of the animals tested) was obtained; 95% confidence limits were calculated after the method of Litchfield et al. (1949). In contrast to the pentylenetetrazol seizure threshold procedure, seizure susceptibility was obtained for each group of animals, rather than for each animal.

The rotarod used in these studies was a one-inch wooden dowel, lightly lacquered to prevent the mice clinging to any cracks or pits in the surface of the wood. The rod was divided by several cardboard disks into sections approximately 15 centimeters wide, and was connected to a variable-speed motor, by means of which it could be made to revolve at any speed desired. In these tests, the rotarod was operated at 15, 20, 25 and 30 rpm. The mice were placed on the rod such that they had to walk forward to remain on the rod, or fall into cages of wood chips placed 50 centimeters below. Mice were placed on the rod as it was moving, and were given three untimed pre-trials, limited to ten seconds each, to acquaint them with the procedure. For the timed trial, mice were timed from the moment they were placed on the rod until the moment they fell off, with a limit of 120 seconds arbitrarily set; any animal remaining on the rod that length of time was removed to avoid overtiring, which could affect subsequent

trials.

Results

Lead appeared to have essentially no effect on pentobarbital sleep time (Table 5-1). This was an unexpected result, in view of the stimulant property of lead on the central nervous system. It is possible that lead, by binding to various drug-metabolizing enzymes, and thereby inactivating them, slightly depressed the metabolism of pentobarbital. This might explain the small increase in sleep time seen when 300 ppm lead was given. It is highly unlikely that central stimulation and enzyme depression exactly balanced each other at the doses of lead given, to result in essentially no net change in sleep time. Subacute administration of 40 mg/kg of lindane daily for two weeks brought about a marked shortening of sleep time which was apparently independent of the presence or absence of lead administration. The decrease was approximately 50% in all cases. In lindane-treated mice, the effect of 300 ppm lead was not the same (compared to the group which received lindane only) as it was in mice not given lindane (compared to controls which received neither lead nor lindane); i. e., no increase, but rather a slight depression, in sleep time occurred. The effect of lindane was apparently so dramatic that any effect lead may have exerted was entirely overshadowed.

Lead exerted no effect on seizure threshold as determined by

Table 5-1. Pentobarbital sleep time in CF #1 mice following administration of lead for eight weeks and of lindane for two weeks.

Level of lead given	Dose of lindane given	
	0	40 mg/kg
	Mean sleep time in minutes ± S. E.	
0	16.38 ± 0.73 (32)[a]	8.52 ± 0.41* (33)
75 ppm	15.88 ± 1.32 (16)	7.88 ± 0.79* (16)
150 ppm	16.38 ± 0.96 (16)	8.56 ± 0.42* (16)
300 ppm	18.24 ± 0.74 (17)	7.76 ± 0.45* (17)

[a]Number of animals.

*Mean significantly different from both the mean of the control group, which received neither lead nor lindane, and from the mean of the group which received the same level of lead but not lindane ($P < .05$).

the pentylenetetrazol seizure threshold test (Tables 5-2 and 5-4). This result, again, was unexpected, due to lead's stimulation of the central nervous system. The results of this test and of the pentobarbital sleep time test indicate either that lead, at the levels given here, does not stimulate the central nervous system of the mouse, or that the stimulation is of such a nature (due perhaps to a specificity in anatomical site of central action) that it cannot be detected by measuring changes in pentobarbital sleep time or in pentylenetetrazol seizure threshold. Lindane raised the pentylenetetrazol seizure threshold significantly regardless of the level of lead administered. Increases in threshold ranged from 35% to 70%. This result was even

Table 5-2. Pentylenetetrazol seizure threshold in CF #1 mice following administration of lead for eight weeks and of lindane for two weeks.

Level of lead given	Dose of lindane given	
	0	40 mg/kg
	Mean time in seconds ± S. E.	
0	47.51 ± 2.82 (32)[a]	70.39 ± 3.75* (33)
75 ppm	45.36 ± 2.93 (16)	61.32 ± 3.31* (16)
150 ppm	47.24 ± 2.96 (18)	74.94 ± 4.20* (17)
300 ppm	47.41 ± 2.06 (16)	80.64 ± 6.59* (16)

[a]Number of animals.

*Mean significantly different from both the mean of the control group, which received neither lead nor lindane, and from the mean of the group which received the same level of lead but not lindane ($P < .05$).

more surprising than the result seen with lead administration. Lindane appeared to stimulate the animals markedly, so a decrease in threshold was anticipated. Possible explanations for this phenomenon are found in the Discussion.

It should be noted that, although a greater dose of pentylenetetrazol was required to elicit a seizure in a lindane-treated animal, the mice experienced tonic seizures and subsequent death rather frequently, whereas mice not given lindane rarely died (Table 5-3). This phenomenon may be worthy of further investigation.

The pattern of pre-seizure activity was quite different in lindane-treated mice compared to mice not given lindane. In those not

Table 5-3. Percent of CF #1 mice experiencing fatal seizures resulting from pentylenetetrazol administration following administration of lead for eight weeks and of lindane for two weeks.

Level of lead given	Dose of lindane given	
	0	40 mg/kg
0	0 (32)[a]	39.4 (33)
75 ppm	0 (16)	37.5 (16)
150 ppm	5.5 (18)	29.4 (17)
300 ppm	0 (16)	50.0 (16)

[a]Number of animals tested.

having received the pesticide, little reaction was observed until a few seconds before the seizure commenced. The first sign was a twitch, the animal immediately assuming an alarm stance, as if suddenly prepared to react quickly to aggression. This stance might be followed by a slight relaxation, the seizure soon supervening. In lindane-treated mice, several of these twitches were observed before the mice succumbed to seizures. The first twitch would usually appear at about the same time as it would in a non-lindane-treated mouse; then would follow relaxation, and resumption of a normal stance. This would be followed by several more twitches and periods of relaxation, which would become more frequent and shortened as the infusion continued. Finally the seizure would begin. And, as was noted in lindane-treated mice, the seizure often progressed to a tonic state, and resulted in death. The possibility is suggested that

lead might make this situation worse with regard to mortality, but this point requires more study.

Acute oral administration of 13 mg/kg of dieldrin (about one-fourth of the acute oral LD50) 36 hours before pentylenetetrazol seizure threshold was determined in lead-treated mice lowered seizure threshold by 22% to 41% (Table 5-4). This result is what had been expected in view of the ability of dieldrin to stimulate the central nervous system. In this test, administration of 300 ppm lead seemed to lower the threshold slightly; when both dieldrin and 300 ppm lead were given, a more distinct depression in seizure threshold occurred.

Table 5-4. Pentylenetetrazol seizure threshold in CF #1 mice following administration of lead for eight weeks and an acute 36-hour pretreatment with dieldrin.

Level of lead given	Dose of dieldrin given	
	0	13 mg/kg
	Mean time in seconds ± S. E.	
0	39.0 ± 1.3 (33)[a]	28.9 ± 1.3* (29)
75 ppm	39.1 ± 1.2 (22)	30.7 ± 1.0* (27)
150 ppm	41.0 ± 1.4 (20)	27.8 ± 1.6* (21)
300 ppm	35.1 ± 2.0 (18)	26.6 ± 1.4* (14)

[a]Number of animals.

*Mean significantly different from both the mean of the control group, which received neither lead nor lindane, and from the mean of the group which received the same level of lead but not lindane ($P < .05$).

Lead showed a tendency to raise low-frequency electroshock seizure susceptibility (Table 5-5). Significant differences were found between the EV50's of the control group and the groups given 75 or 150 ppm lead. This increase in susceptibility may be a reflection of the stimulation of lead in the central nervous system. Lindane caused a significant decrease in seizure susceptibility (an average of 23%) both in the presence and in the absence of lead intoxication. This decrease parallels that seen in the pentylenetetrazol seizure threshold test (expressed as an increase in seizure threshold), and was equally unexpected. Possible explanations are given in the Discussion.

Table 5-5. Low-frequency electroshock seizure median effective voltages in CF #1 mice following administration of lead for eight weeks and of lindane for two weeks.

Level of lead given	Dose of lindane given	
	0	40 mg/kg
	EV50 (95% confidence limits)	
0	31.0 (29.1-33.0) (27)[a]	35.5 (32.9-38.3)** (23)
75 ppm	28.0 (26.8-29.3)* (28)	35.0 (33.6-36.5)** (19)
150 ppm	26.5 (24.3-28.9)* (20)	35.0 (32.1-38.2)** (22)
300 ppm	29.0 (26.6-31.6) (20)	35.0 (33.6-36.4)** (22)

[a] Number of animals.

* Mean significantly different from mean of control group as in Table 4-1 ($P < .05$).

** Mean significantly different from both the mean of the control group, which received neither lead nor lindane, and from the mean of the group which received the same level of lead but not lindane ($P < .05$).

Lead administration affected rotarod performance only occasionally. Table 5-6 and Figure 5-1 indicate the usual effect of lead administration observed in several trials. The results shown in Table 5-8 indicate that lead decreased the time mice remained on the rotarod; but such results were observed approximately 10% of the time, and equally often the inverse was observed. These effects seemed to occur randomly. It was thought that perhaps the reason definite increases or decreases in time spent on the rotarod were not seen was that the levels of lead being given were not great enough. So several experiments (not illustrated here) were performed on mice receiving 600 ppm, 1200 ppm, 2400 ppm and 4800 ppm lead. Some of these experiments were performed after the mice had been receiving lead for periods of up to six months. The results of these experiments were generally identical to those seen in Table 5-6. Variability was a problem, as it was quite large within each experiment, and of some magnitude from experiment to experiment. Controls varied between experiments by a factor of ten or more in time spent on the rotarod. Comparable variation was seen in lead-treated animals. Lead in some cases appeared to decrease rotarod performance, and at other times it appeared to increase it or leave it unaffected.

Chronic administration of dieldrin at levels of 0.5 ppm and 1.0 ppm in food resulted in decreased ability of mice to remain on the rotarod (Table 5-7 and Figure 5-2). Chronic administration of dieldrin

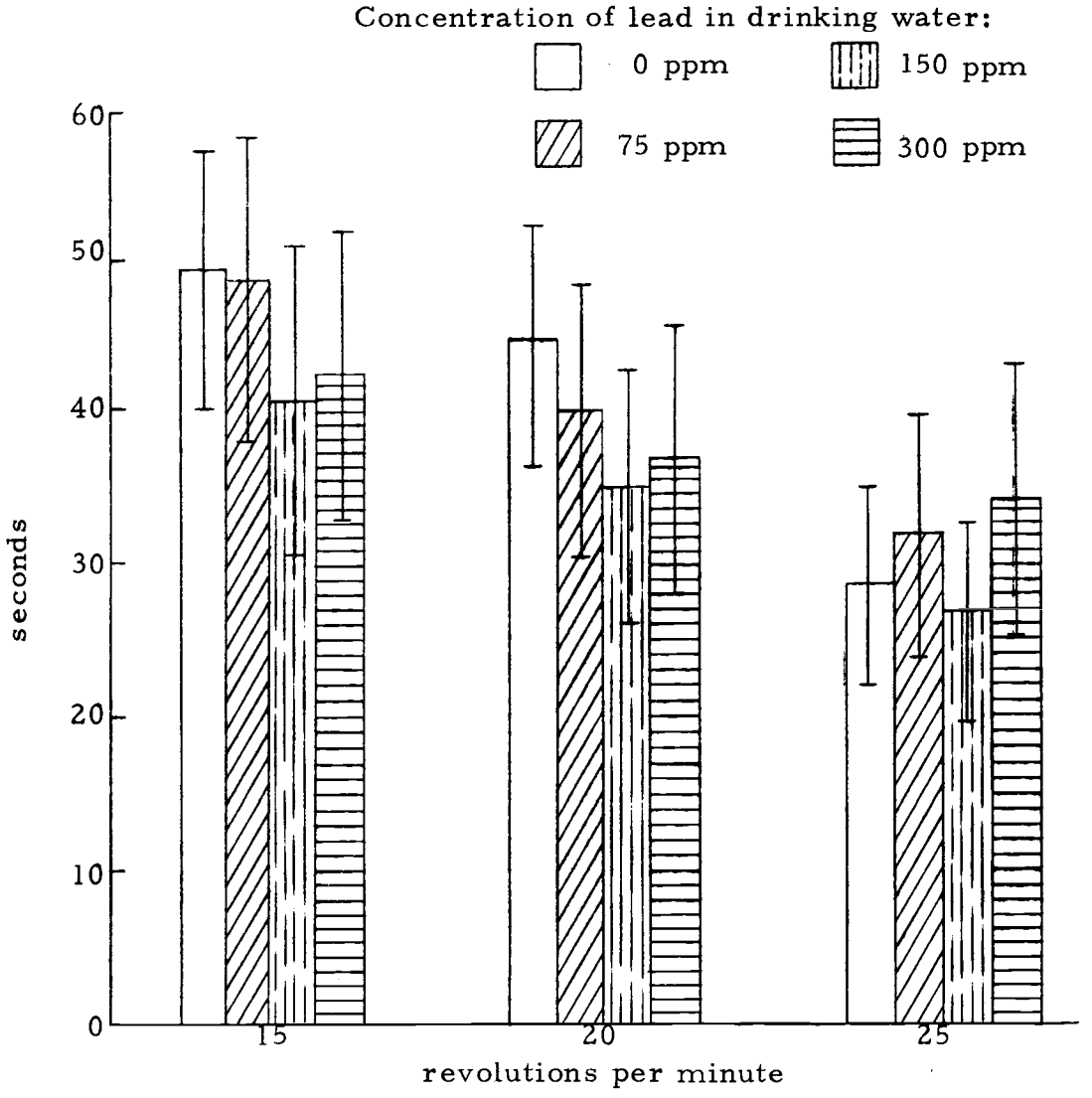

Figure 5-1. Length of time CF #1 mice remained on the rotarod following administration of lead for eight weeks. Bars are means; vertical lines are standard errors.

Table 5-6. Length of time CF #1 mice remained on the rotarod following administration of lead for eight weeks.

rpm of rotarod	Level of lead given (ppm)			
	0 (22)[a]	75 (21)	150 (22)	300 (18)
	Mean time in seconds ± S. E.			
15	49.6 ± 8.5	49.1 ± 10.4	41.1 ± 10.0	43.1 ± 9.1
20	44.5 ± 8.2	40.1 ± 9.3	35.1 ± 8.3	37.3 ± 8.8
25	29.0 ± 6.4	32.2 ± 7.6	26.7 ± 6.4	34.9 ± 8.6

[a]Number of animals.

Table 5-7. Length of time CF #1 mice remained on the rotarod following administration of dieldrin in food for four weeks.

rpm of rotarod	Level of dieldrin given (ppm)		
	0 (14)[a]	0.5 (24)	1.0 (24)
	Mean time in seconds ± S. E.		
15	64.6 ± 14.1	51.2 ± 9.3	30.6 ± 8.6*
20	52.0 ± 12.0	39.1 ± 7.4	14.0 ± 2.9*
25	31.9 ± 10.0	31.3 ± 6.8	12.7 ± 3.7*
30	34.4 ± 10.0	19.6 ± 4.2	7.6 ± 2.0*

[a]Number of animals.

*Mean significantly different from control mean, as in Table 4-1 ($P < .05$).

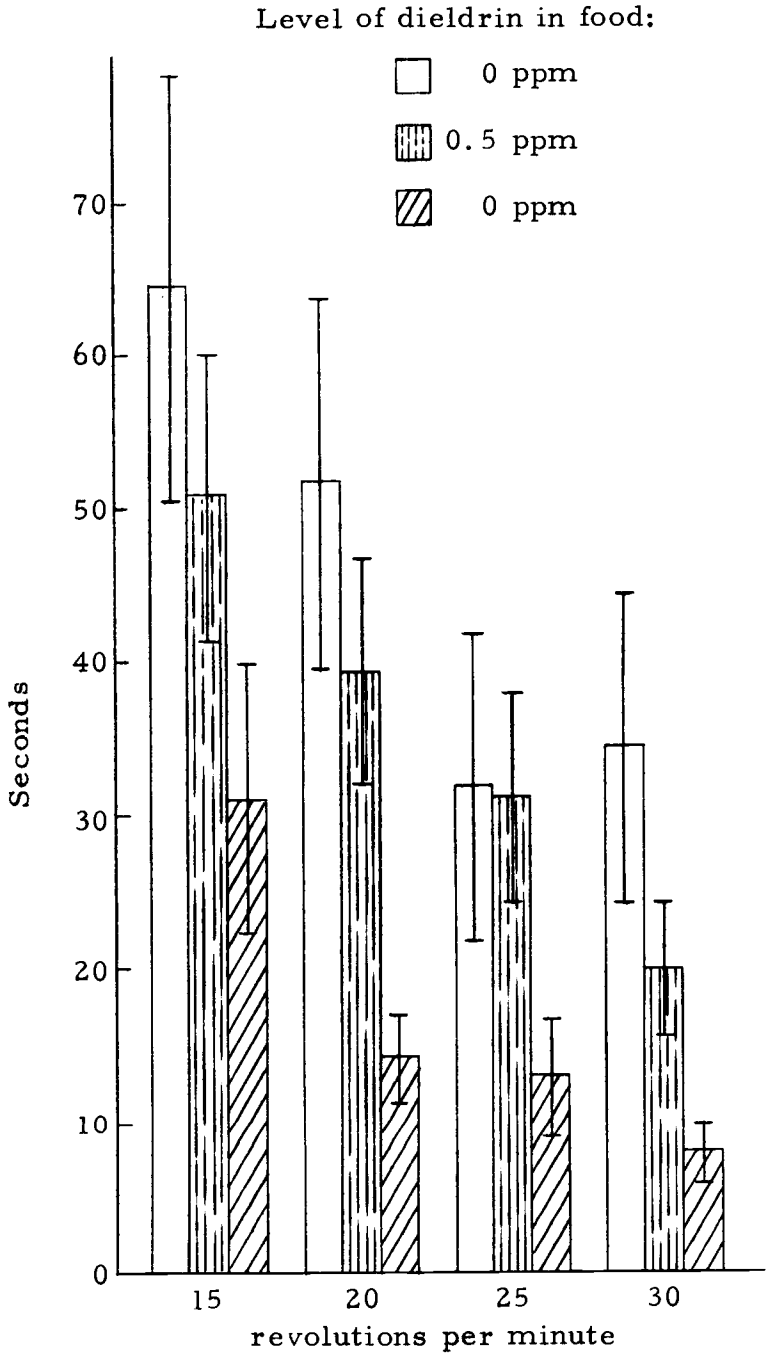

Figure 5-2. Length of time CF #1 mice remained on the rotarod following administration of dieldrin in food for four weeks. Bars are means; vertical lines are standard errors.

in concentrations of up to 2.5 ppm resulted in only slight increases in activity indicative of central excitation. There was some increase in fighting among the mice, and a tendency to avoid being picked up. Bone and Harr (1966) observed central hyperexcitability in rats fed diets containing dieldrin at levels of from 1.0 ppm to 5.0 ppm. Microscopic studies by these workers revealed central histologic damage.

When both lead and dieldrin were given chronically, the effect was that shown in Table 5-8. Although lead intoxication tended to decrease the length of time a mouse spent on the rotarod and dieldrin by itself elicited the same response, the agents, when administered concomitantly, appeared to antagonize each other. Little weight will be given any of these results because of the small numbers of animals used.

Table 5-8. Length of time CF #1 mice remained on the rotarod (25 rpm) following administration of lead for six weeks and of dieldrin for four weeks.

Level of lead given	Level of dieldrin given	
	0	2.5 ppm
	Mean time in seconds ± S. E.	
0	49.3 ± 17.7 (6)[a]	26.2 ± 17.0 (6)
38 ppm	22.1 ± 6.9 (8)	29.2 ± 16.6 (6)
75 ppm	9.2 ± 5.3* (6)	15.3 ± 11.7 (4)

[a]Number of animals.
*Mean significantly different from control mean, as in Table 4-1 ($P < .05$).

Discussion

The termination of pentobarbital-induced sleep is dependent upon redistribution of the drug, the rate of metabolism of the drug (which can be altered pharmacologically), and the state of activity of the central nervous system at the time of drug administration (Kato, 1959a, b, 1960a, b; Kato and Chiesara, 1962; Kato, Chiesara and Vassanelli, 1962). Whereas it is known that lead stimulates the central nervous system, the degree of stimulation or the type of stimulation elicited by the levels of lead given to this species in these tests was almost too small to be measured by the pentobarbital sleep time test. Evidently neither the rate of redistribution nor the rate of metabolism of the pentobarbital was altered by lead, despite the dramatic depression of levels of cytochrome P-450, which would presumably result in a depression of barbiturate metabolism.

On the other hand, a pronounced effect on sleep time was observed with lindane administration. This was expected, as lindane is a well-known stimulant of the central nervous system (Dreisbach, 1963). In these studies, marked stimulation of mice was a constant finding. Sleep time may have been shortened by this effect. But in addition, sleep time may well have been shortened by a mechanism related to enzyme induction, as in the studies of Kato (1959a, b, 1960a, b). If pentobarbital metabolism were accelerated, the effects of the drug would diminish more quickly. This is probably the principal

reason for the decrease in sleep time observed in these studies.

The lethal effect of lindane in the pentylenetetrazol seizure test is reminiscent of a chemical reaction wherein a certain energy of activation must be added to a system before the reaction can occur. In other words, the seizure threshold seems to resemble an energy threshold which must be exceeded before the seizure can take place. After this threshold energy is attained, however, the reaction may cause the release of energy in excess of that of the threshold. Likewise, the central "energy" released after the seizure threshold is exceeded could be greater than the sum of the "endogenous" central activity (energy) and the "exogenous central energy" supplied by the stimulant drug, pentylenetetrazol. This amount of "central energy" results in lethal hyperexcitation of the central nervous system.

It is also likely that in the lindane-treated animals the minimal seizure was suppressed to a point where it was not recognized as such. In such a case, pentylenetetrazol infusion would continue until a tonic seizure, which was not suppressed to the same extent as the minimal seizure, occurred. Such suppression of a minimal seizure might have been brought about by an adaptation of the animal to the constant stimulation of lindane exposure. The level of lindane given was of insufficient magnitude to induce seizures, but was apparently capable of bringing about an adaptation of the animal to minimal seizure-induced stimulation.

The decrease of pentylenetetrazol seizure threshold by an acute administration of dieldrin was not unexpected, in view of the central stimulant property of the pesticide. In contrast to the effect of subacute lindane administration, acute dieldrin administration did not result in an increase in the percent of mice experiencing tonic seizures nor in the lethality of the mice subjected to the test. The reason for this difference may have been the difference in the dose schedules; i.e., lindane was given subacutely, and dieldrin was given acutely.

Lead administration did not appear to affect central activity as measured by pentobarbital sleep time or pentylenetetrazol seizure threshold determination. Perhaps changes in central activity are more difficult to measure in the mouse than in man or some other species. The results of the ALA and coproporphyrin analyses, as well as the cytochrome P-450 study, indicate that lead was definitely absorbed in toxic amounts; and low-frequency electroshock seizure susceptibility was raised by lead administration. However, the levels of lead used and the time period employed were apparently of insufficient magnitude to produce observable central changes as measured by any of the tests of central activity employed in this study, except the low-frequency electroshock seizure susceptibility determination.

Administration of lindane, on the other hand, showed definite effects on central activity. The pentylenetetrazol seizure threshold

was raised, low-frequency electroshock susceptibility was lowered, and pentobarbital sleep time was decreased. The decreases in seizure susceptibilities were due to a central effect, whereas the decrease in pentobarbital sleep time was likely a biochemical effect, as mentioned previously. The central effect appears to consist of depression, at least of those centers acted upon by pentylenetetrazol and electroshock. Since lindane is considered to be a central stimulant, this depressant effect may have been caused by one or more metabolites of lindane, such as 2, 3, 5-trichlorophenyl glucosiduronic acid or 2, 4, 5-trichlorophenol (Grover and Sims, 1965). Lead administration did not appear to alter the effect of lindane.

The effects of dieldrin on the central nervous system seemed to consist of simple stimulation. This stimulation was manifest as a decrease in pentylenetetrazol seizure threshold. No interaction between lead and dieldrin was observed.

Because of the large variability of the results obtained with the rotarod test, no definite conclusions can be drawn. The variable results did not seem to be dependent on the time of day nor on the season of the year; temperature was probably not a factor, as all tests were performed in a laboratory in which the temperature was kept constant. The variability is underscored by the differences found for control animals from experiment to experiment. The only conclusion that can be drawn from this study is that this test, as performed here,

is not a reasonable indicator of lead intoxication in mice.

Chronic intoxication with dieldrin was demonstrated by the rotarod test quite easily. Perhaps this is because dieldrin may affect fewer systems than does lead; thus, if the only variable being altered were, for instance, the degree of ataxia, other effects would not interfere. Cnly when chronic lead intoxication was superimposed upon chronic dieldrin intoxication was the rotarod test rendered less effective in determining dieldrin poisoning.

BIBLIOGRAPHY

Albahary, C. 1964. Les troubles prophyriques dans le saturnisme. Etude comparèe à propos de 33 malades hospitalisés. Archives des Maladies Professionelles, de Medecine du Travail et de Securite Sociale 25:495-507. (Abstracted in Biological Abstracts 46:61812. 1965)

Altman, Philip L. and Dorothy S. Dittman (eds.). 1964. Biology data book. Washington, D. C., Federation of American Societies for Experimental Biology. 633 p.

American Medical Association. 1962. Registry on blood dyscrasias. Report to the council. The Journal of the American Medical Association 179:888-890.

Artz, Curtis P. 1941. Lead intoxication in children from the burning of battery casings; report of two cases. West Virginia Medical Journal 37:410-414. (Abstracted in Biological Abstracts 16: 16379. 1942)

Ashe, W. F. 1943. Industrial lead poisoning as a clinical syndrome. Journal of Industrial Hygiene and Toxicology 25:55-59.

Aub, Joseph C. 1935. Biochemical behavior of lead in the body. The Journal of the American Medical Association 104:87-90.

Aub, Joseph C., L. T. Fairhall, A. S. Minot and P. Reznikoff. 1925. Lead poisoning. Medicine 4:1-250.

_____ 1926. Lead poisoning. Medicine monographs. Vol. 7. Baltimore, Williams and Wilkins. 273 p.

Bacon, A. P. C., K. Froome, A. E. Gent, T. K. Cooke and P. Sowerby. 1967. Lead poisoning from drinking soft water. The Lancet, 1967, Vol. 1, p. 264-266.

Baier, Helmut. 1958a. Über die Wirkung von blei auf die Acetylcholinsynthese. Klinische Wochenschrift 36:681-682. (Abstracted in Chemical Abstracts 53:9474i. 1959)

_____ 1958b. Über die Wirkung von blei auf die Fermentsynthese in vivo. Klinische Wochenschrift 36:970-972. (Abstracted in Chemical Abstracts 53:12478a. 1959)

Barltrop, Donald. 1966. The prevalence of pica. American Journal of Diseases of Children 112:116-123.

Baron, J. and T. R. Tephly. 1969. Effect of 3-amino-1, 2, 4-triazole on the stimulation of hepatic microsomal heme synthesis and induction of hepatic microsomal oxidases produced by phenobarbital. Molecular Pharmacology 5:10-20.

Berkson, Joseph, Thomas B. Magath and Margaret Hurn. 1940. The error of estimate of the blood cell count as made with the hemocytometer. The American Journal of Physiology 128:309-323.

Bone, J. F. and J. R. Harr. 1966. Unpublished research on dieldrin intoxication. Corvallis, Oregon, Oregon State University, Department of Veterinary Medicine.

Bracken, E. C., D. E. Beaver and C. C. Randall. 1958. Histochemical studies of viral and lead-induced intranuclear bodies. The Journal of Pathology and Bacteriology 75:253-256.

Brief, Richard S. 1962. Air lead concentrations from automotive engines. Archives of Environmental Health 5:527-531.

Brown, John E. and Earl E. Smith. 1937. Peripheral neuritis due to lead. The Journal of Pediatrics 10:656-665.

Brown, W. C., D. O. Schiffman, E. A. Swinyard and L. S. Goodman. 1953. Comparative assay of antiepileptic drugs by "psychomotor" seizure test and minimal electroshock threshold test. The Journal of Pharmacology and Experimental Therapeutics 107:273-283.

Bullock, John D., Robert J. Wey, John A. Zaia, Irwin Zarembok and Henry A. Schroeder. 1966. Effect of tetraethyllead on learning and memory in the rat. Archives of Environmental Health 13:21-22.

Byers, R. K. 1959. Lead poisoning: Review of the literature and report on 45 cases. Pediatrics 23:585-603.

Castellino, N. and S. Aloj. 1964. Distribution and excretion of lead-210 in the rat. Folia Medica 47:138-156. (Abstracted in Chemical Abstracts 61:13796b. 1964)

Catizone, Olga and Peter Gray. 1941. Experiments on chemical interference with the early morphogenesis of the chick. II. The effects of lead on the central nervous system. The Journal of Experimental Zoology 87:71-83.

Chisolm, J. Julian. 1964. Disturbances in the biosynthesis of heme in lead intoxication. The Journal of Pediatrics 64:174-187.

Conney, A. H. 1967. Pharmacological implications of microsomal enzyme induction. Pharmacological Reviews 19:317-366.

Conney, A. H., I. A. Michaelson and J. J. Burns. 1961. Stimulatory effect of chlorcyclizine on barbiturate metabolism. The Journal of Pharmacology and Experimental Therapeutics 132:202-206.

Conney, A. H., E. C. Miller and J. A. Miller. 1956. The metabolism of methylated aminoazo dyes. V. Evidence for induction of enzyme synthesis in the rat by 3-methylcholanthrene. Cancer Research 16:450-459.

Cooper, David Y., Sidney Levin, Shakunthala Narasimhulu, Otto Rosenthal and Ronald W. Estabrook. 1965. Photochemical action spectrum of the terminal oxidase of mixed function oxidase systems. Science 147:400-402.

Cooper, George. 1947. An epidemic of inhalation lead poisoning with characteristic skeletal changes in the children involved. The American Journal of Roentgenology and Radium Therapy 58: 129-141.

Copeman, P. R. van der Reit. 1945. The presence of copper and lead in some human tissues. Clinical Proceedings 4:473-482. (Abstracted in Biological Abstracts 20:16972. 1946)

Cotter, Lawrence H. 1946. Lead intoxication by inhalation. Journal of Industrial Hygiene and Toxicology 28:44-46.

Crawford, M. D. and J. N. Morris. 1967. Lead in drinking water. The Lancet, 1967, Vol. 2, p. 1087-1088.

Cremer, Jill E. 1961. Toxicity of tetraethyllead and related alkyl metallic compounds. Annals of Occupational Hygiene 3:226-230. (Abstracted in Chemical Abstracts 61:7583f. 1964)

Dale, E., M. F. Copeland and W. J. Hayes. 1965. Chlorinated insecticides in the body fat of people in India. Bulletin of the World Health Organization 33:471-477.

Dale, E. and Griffeth E. Quinby. 1963. Chlorinated insecticides in the body fat of people in the United States, Science 142:593-595.

Dalldorf, Gilbert and R. R. Williams. 1945. Impairment of reproduction in rats by ingestion of lead. Science 102:668-670.

Daubenspeck, G. Walker, Arvid Teinson and Alfred M. Noyes. 1944. Industrial-hygiene survey of soldering operations. Illinois Department of Labor, Division of Factory Inspection, Technical Paper no. 5. 25 p. (Abstracted in Chemical Abstracts 39: 4313-1. 1945)

Davis, Joseph R. and Samuel L. Andelman. 1967. Urinary delta-aminolevulinic acid (ALA) levels in lead poisoning. Archives of Environmental Health 15:53-59.

DeKretser, A. J. and H. A. Waldron. 1963. Urinary delta-aminolevulinic acid and porphobilinogen in lead-exposed workers. British Journal of Industrial Medicine 20:35-40. (Abstracted in Biological Abstracts 44:6575. 1963)

Del Castillo-Nicolau, J. and H. J. Hufschmidt. 1951. Reversible poisoning of nerve fibres by heavy-metal ions. Nature 167: 146-147.

DeMatteis, F., B. E. Prior and C. Rimington. 1961. Nervous and biochemical disturbances following hexachlorobenzene intoxication. Nature 191:363-366.

DeMello, R. Pimenta. 1951. Effect of light on urinary coproporphyrin excretion in lead-poisoned rabbits. Proceedings of the Society for Experimental Biology and Medicine 76:823-825.

Detection of industrial lead poisoning. 1966. The Lancet, 1966, Vol. 1, p. 191-192.

Dreessen, W. C. 1943. Health of lead-exposed storage-battery workers. Journal of Industrial Hygiene and Toxicology 25: 60-70.

Dreisbach, Robert H. 1963. Handbook of poisoning. 4th ed. Los Altos, California, Lange Medical Publications. 467 p.

Dunham, N. W. and T. S. Miya. 1957. A note on a simple apparatus for detecting neurological deficit in rats and mice. Journal of the American Pharmaceutical Association, scientific ed., 46: 208-209.

Duvoir, M., L. Dérobert and A. Hadengue. 1947. La granulobasophilia, test temporaire au cours de l'intoxication saturnine experimentale. Archives ces Maladies Professionelles, de Medecine du Travail et de Securite Sociale 8:1-3. (Abstracted in Biological Abstracts 23:21261. 1949)

Egan, H., R. Goulding, J. Roburn and J. O'G. Tatton. 1965. Organochlorine pesticide residues in human fat and human milk. British Medical Journal, 1965, Vol. 2, p. 66-69.

Ehrlich, George E. and John Chokatos. 1966. Saturnine gout. Archives of Internal Medicine 118:572-574.

Estabrook, Ronald W., David Y. Cooper and Otto Rosenthal. 1963. The light reversible carbon monoxide inhibition of the steroid C 21-hydroxylase system of the adrenal cortex. Biochemische Zeitschrift 338:741-755.

Fairhall, Lawrence T. 1943. The identification and localization of lead in bone tissue. Public Health Reports 58:209-216.

Feigin, Ralph D., Daniel C. Shannon, Stephen L. Reynolds, Lilian W. Shapiro and John P. Connelly. 1965. Lead poisoning in children. Clinical Pediatrics 4:38-45.

Ferm, Vergil H. and Stanley J. Carpenter. 1967. Developmental malformations resulting from the administration of lead salts. Experimental and Molecular Pathology 7:208-213.

Fingl, E. and D. G. McQuarrie. 1960. Evaluation of variables for the "timed intravenous infusion" procedure for pentylenetetrazol seizures in mice. Archives Internationales de Pharmacodynamie et de Thérape 126:17-30.

Finner, Lucy L. and Herbert O. Calvery. 1939. Pathologic changes in rats and dogs fed diets containing lead and arsenic compounds. Archives of Pathology 27:433-446.

Frankel, S. and S. Reitman (eds.). 1963. Gradwohl's clinical laboratory methods and diagnosis. 6th ed. St. Louis, C. V. Mosby. 2092 p.

Garfinkel, David. 1958. Studies on pig liver microsomes. I. Enzymic and pigment composition of different microsomal fractions. Archives of Biochemistry and Biophysics 77:493-509.

George, W. J. and T. R. Tephly. 1968. Studies on hepatic microsomal N- and O-dealkylation of morphine analogues. Molecular Pharmacology 4:502-509.

Giel, Charles P., Morris Kleinfeld and Jacqueline Messite. 1956. Lead toxicity in a storage-battery plant. A. M. A. Archives of Industrial Health 13:321-325.

Gilfillan, S. C. 1965. Lead poisoning and the fall of Rome. Journal of Occupational Medicine 7:53-60.

Gillett, James W. and Timothy M. Chan. 1969. Unpublished research on enzyme induction. Corvallis, Oregon, Oregon State University, Department of Agricultural Chemistry.

Gillette, J. R., J. J. Kamm and H. A. Sasame. 1968. Mechanism of p-nitrobenzoate reduction in liver: the possible role of cytochrome P-450 in liver microsomes. Molecular Pharmacology 4:541-548.

Glotfelty, James S. 1942. Psychosis following lead arsenate poisoning. The Medical Bulletin of the Veteran's Administration 18:334-335.

Gocher, T. E. 1945. Plumbism. Northwest Medicine 44:283-286.

Goldsmith, John R. and Alfred C. Hexter. 1967. Respiratory exposure to lead: Epidemiological and experimental dose-response relationships. Science 158:132-134.

Goldwater, Leonard J. 1967. "Normal" concentrations of metals in urine and blood. WHO Chronical 21:191-192.

Goodman, L. S., J. E. P. Toman and E. A. Swinyard. 1949. Anticonvulsant drugs: mechanisms of action and methods of assay. Archives Internationales de Pharmacodynamie et de Thérapie 78:144-162.

Gordon, Neil, E. King and R. I. Mackay. 1967. Lead absorption in children. British Medical Journal, 1967, Vol. 2, p. 480-482.

Granick, S. 1966. The induction in vitro of the synthesis of delta-aminolevulinic acid synthetase in chemical porphyria: A response to certain drugs, sex hormones and foreign chemicals. The Journal of Biological Chemistry 241:1359-1375.

Granick, S. and Gumpei Urata. 1963. Increase in activity of delta-aminolevulinic acid synthetase in liver mitochondria induced by feeding of 3,5-dicarbethoxy-1,4-dihydrocollidine. The Journal of Biological Chemistry 238:821-827.

Greengard, Joseph. 1966. Lead poisoning in childhood: Signs, symptoms, current therapy, clinical expressions. Clinical Pediatrics 5:269-276.

Grover, P. L. and P. Sims. 1965. The metabolism of gamma-2,3,4,5,6-pentachlorocyclohex-1-ene and gamma-hexachlorocyclohexane in rats. Biochemical Journal 96:521-525.

Guarino, A. M., T. E. Gram, P. L. Gigon, F. E. Greene and J. R. Gillette. 1969. Changes in Michaelis and spectral constants for aniline in hepatic microsomes from phenobarbital-treated rats. Molecular Pharmacology 5:131-136.

Haley, Thomas J. 1966. Chronic lead intoxication from environmental contamination: Myth or fact? Archives of Environmental Health 12:781-785.

Halley, James W. 1941. Atmospheric concentration of lead fume associated with forging, welding, and oxygen cutting of lead-bearing steel, based upon experimental studies. Journal of Industrial Hygiene and Toxicology 23:100-105.

Hardy, Harriet L. 1966. What is the status of knowledge of the toxic effect of lead on identifiable groups in the population? Clinical Pharmacology and Therapeutics 7:713-722.

Harris, Robert W. and William R. Elsea. 1967. Ceramic glaze as a source of lead poisoning. The Journal of the American Medical Association 202:544-546.

Hart, L. G. and J. R. Fouts. 1965. Further studies on the stimulation of hepatic microsomal drug-metabolizing enzymes by DDT and its analogs. Naunyn-Schmiedebergs Archiv für Experimentelle Pathologie und Pharmakologie 249:486-500.

Harvey, Stewart C. 1965. Heavy metals. In: The pharmacological basis of therapeutics, ed. by Louis S. Goodman and Alfred Gilman. 3d ed. New York, Macmillan. p. 943-975.

Hass, George W., Wayne Landerholm and Anne Hemmens. 1967. Inhibition of intercellular matrix synthesis during ingestion of inorganic lead. The American Journal of Pathology 50:815-847.

Hayes, W. J. 1965. Insecticides, rodenticides, and other economic poisons. In: Drill's pharmacology in medicine, ed. by Joseph R. DiPalma. 3d ed. New York, McGraw-Hill. p. 989-1004.

Hayes, W. J. and W. E. Dale. 1963. Storage of insecticides in French poeple. Nature 199:1189-1191.

Hayes, W. J., W. E. Dale and Virlyn W. Burse. 1965. Chlorinated hydrocarbon pesticides in the fat of people in New Orleans. Life Sciences 4:1611-1615.

Hoffman, W. S., Howard Adler, W. I. Fishbein and Frank C. Bauer. 1967. Relation of pesticide concentrations in fat to pathological changes in tissues. Archives of Environmental Health 15: 758-765.

Hoffman, W. S., W. I. Fishbein and M. B. Andelman. 1964a. The pesticide content of human fat tissue. Archives of Environmental Health 9:387-394.

_____ 1964b. Pesticide storage in human fat. The Journal of the American Medical Association 188:819.

Holmstedt, B. and C. Liljestrand. 1963. Readings in pharmacology. New York, Macmillan. 395 p.

Holtzman, J. L., T. E. Gram, P. L. Gigon and J. R. Gillette. 1968. The distribution of the components of mixed function oxidase between the rough and the smooth endoplasmic reticulum of liver cells. Biochemical Journal 110:407-412.

Horiuchi, Kazuya, Hirokazu Noma, Isao Asano and Kenji Hashimoto. 1962. Industrial lead poisoning. An experimental study of lead intake in human beings through the respiratory tract. Osaka City Medical Journal 8:151-169. (Abstracted in Chemical Abstracts 61:2384h. 1964)

Hughes, James P. 1965. Lead and chelating agents. In: Drill's pharmacology in medicine, ed. by Joseph R. DiPalma. 3d ed. New York, McGraw-Hill. p. 845-859.

Hunter, C. G., J. Robinson and A. Richardson. 1963. Chlorinated hydrocarbon insecticide content of human body fat in southern England. British Medical Journal, 1963, Vol. 1, p. 221-224.

Hunter, C. G., J. Robinson and M. Roberts. 1969. Pharmacodynamics of dieldrin (HEOD). Archives of Environmental Health 18:12-21.

Jacobziner, Harold and Harry W. Raybin. 1962. The epidemiology of lead poisoning in children. Archives of Pediatrics 79:72-76.

Joliff, Carl R., Paul J. Heidrick, Jerome A. Cain and John E. Ohlsen. 1959. Lead in domestic water supply. Lead piping as a source of toxic quantities. Nebraska State Medical Journal 44:156-160. (Abstracted in Chemical Abstracts 54:7011f. 1960)

Kato, R. 1959a. Un pretrallamento, eseguito 48 ore prima, con svariate sostanze puo diminuire gli effetti farmacologici del pentobarbital. Atti della Societa Lombarda di Scienze Mediche e Biologiche 14:777-780. (Cited in: Conney, A. H. and J. J. Burns. 1962. Factors influencing drug metabolism. In: Advances in pharmacology, ed. by Silvio Garattini and Parkhurst A. Shore. Vol. 1. New York, Academic Press. p. 31-58)

_____ 1959b. Su alcuni caratteri della diminuzione di sensibilita al pentobarbital in animali pretrattati con fenaglicodolo. Atti della Societa Lombarda di Scienze Mediche e Biologiche 14:781-783. (Cited in: Conney, A. H. and J. J. Burns. 1962. Factors influencing drug metabolism. In: Advances in pharmacology, ed. by Silvio Garattini and Parkhurst A. Shore. Vol. 1. New York, Academic Press. p. 31-58)

_____ 1960a. Induced increase of meprobamate metabolism in rats with phenobarbital or phenaglycodol. Medica Experimentalis 3:95-100. (Cited in: Conney, A. H. and J. J. Burns. 1962. Factors influencing drug metabolism. In: Advances in pharmacology, ed. by Silvio Garattini and Parkhurst A. Shore. Vol. 1. New York, Academic Press. p. 31-58)

_____ 1960b. Reduced sensitivity to some drugs (barbiturates) forty-eight hours after chlorpromazine treatment. Experientia 16:427-428.

Kato, R. and E. Chiesara. 1962. Increase of phenobarbitone metabolism induced in rats pretreated with some centrally acting compounds. British Journal of Pharmacology and Chemotherapy 18:29-38.

Kato, R., E. Chiesara and P. Vassanelli. 1962. Factors influencing induction of hepatic microsomal drug-metabolizing enzymes. Biochemical Pharmacology 11:211-220.

Kato, R. and A. Takahashi. 1968. Thyroid hormone and activities of drug-metabolizing enzymes and electron transport systems of rat liver microsomes. Molecular Pharmacology 4:109-120.

Kehoe, Robert A. 1943. Lead absorption and lead poisoning. Medical Clinics of North America 26:1261-1279.

Kehoe, Robert A., Robert V. Story, Jacob Cholak, Donald M. Hubbard, Karl Bambach and Robert M. McNary. 1940. Experimental studies on the ingestion of lead compounds. Journal of Industrial Hygiene and Toxicology 22:381-400.

Kehoe, Robert A., F. Thamann and J. Cholak. 1933. On normal absorption and excretion of lead; lead absorption and lead excretion in modern American life. Journal of Industrial Hygiene and Toxicology 15:273-288.

Klingenberg, Martin. 1958. Pigments of rat liver microsomes. Archives of Biochemistry and Biophysics 75:376-386.

Konopinski, Virgil J. and James B. Upham. 1967. Commuter exposure to atmospheric lead. Archives of Environmental Health 14:589-593.

Kostial, Krista and V. B. Vouk. 1957. Lead ions and synaptic transmission in the superior cervical ganglion of the cat. British Journal of Pharmacology and Chemotherapy 12:219-222.

Kreimer-Birnbaum, Martha and Moisés Grinstein. 1965. Porphyrin biosynthesis. III. Porphyrin metabolism in experimental lead poisoning. Biochemica et Biophysica Acta 111:110-123.

Lead poisoning in children. 1964. British Medical Journal, 1964. Vol. 1, p. 1200-1201.

Lehmann, K. B. 1924. Experimentelle Beitrage zum Studium der chronischen Bleivergiftung. Archiv für Hygiene 94:1-40.

Litchfield, J. T. and F. Wilcoxon. 1949. A simplified method for evaluating dose-effect experiments. The Journal of Pharmacology and Experimental Therapeutics 96:99-113.

Loge, J. Philip. 1965. Aplastic anemia following exposure to benzene hexachloride (lindane). The Journal of the American Medical Association 193:110-114.

Lowry, Oliver H., Nira J. Rosebrough, A. Lewis Farr and Rose J. Randall. 1951. Protein measurement with the folin phenol reagent. The Journal of Biological Chemistry 193:265-275.

McGill, A. E. J. and J. Robinson. 1968. Organochlorine insecticide residues in complete prepared meals: A 12-month survey in S. E. England. Food and Cosmetics Toxicology 6:45-57.

McNiel, J. R. and M. C. Reinhard. 1967. Lead poisoning from home remedies. Clinical Pediatrics 6:150-156.

Mao, Peter and John J. Molnar. 1967. The fine structure and histochemistry of lead-induced renal tumors in rats. The American Journal of Pathology 50:571-603.

Marver, Harvey S., Annie Collins, Donald P. Tschudy and Miloslav Rechcigl. 1966. Delta-ALA synthetase. II. Induction in rat liver. The Journal of Biological Chemistry 241:4323-4329.

Mehani, Shawkia. 1966. Lead retention by the lungs of lead-exposed workers. Annals of Occupational Hygiene 9:165-171. (Abstracted in Biological Abstracts 47:118434. 1966)

Moeschlin, Sven. 1965. Poisoning; diagnosis and treatment, tr. by Jenifer Bickel from the 4th German ed. New York, Grune and Stratton. 707 p.

Mumma, Ralph O., Willis B. Wheeler, Donald E. H. Frear and Robert H. Hamilton. 1966. Dieldrin: Extraction of accumulations by root uptake. Science 152:530-531.

Nachmansohn, D. and E. Lederer. 1939. Sur quelques proprietes chimiques de la cholinesterase. Comptes Rendus des Seances de la Societe de Biologie et de Ses Filiales 120:321-324. (Cited in: Sávay, G. and B. Csillik. 1958. Lead reactive substances

in peripheral synapses. Experientia 15:396-397)

Negherbon, William O. 1959. Insecticides. Philadelphia, W. B. Saunders. 854 p. (Handbook of toxicology, Vol. 3)

Ockner, Robert K. and Rudi Schmid. 1961. Acquired porphyria in man and rat due to hexachlorobenzene intoxication. Nature 189:499.

Odescalchi, C. P. and P. Andreuzzi. 1959. Capillary resistance in subacute lead poisoning. Experiments in rats. Folia Medica 42:111-131. (Abstracted in Chemical Abstracts 53:14335a. 1959)

Omura, T. and R. Sato. 1963. Fractional solubilization of haemoproteins and partial purification of carbon monoxide-binding cytochrome from liver microsomes. Biochemica et Biophysica Acta 71:224-226.

_____ 1964. The carbon monoxide-binding pigment of liver microsomes. The Journal of Biological Chemistry 239:2370-2378.

Omura, T., R. Sato, D. Y. Cooper, O. Rosenthal and R. W. Estabrook. 1965. Function of cytochrome P-450 of microsomes. Federation Proceedings 24:1181-1189.

Orrenius, S. and L. Ernster. 1964. Phenobarbital-induced synthesis of the oxidative demethylating enzymes of rat liver microsomes. Biochemical and Biophysical Research Communications 16:60-65.

Orrenius, S., G. Gallner and L. Ernster. 1964. Inhibition of the TPNH-linked lipid peroxidation of liver microsomes by drugs undergoing oxidative demethylation. Biochemical and Biophysical Research Communications 14:329-334.

Parry, Wilfrid H. 1967. Lead in drinking water. The Lancet, 1967, Vol. 2, p. 1207-1208.

Patterson, Clair C. 1965. Contaminated and natural lead environments of man. Archives of Environmental Health 11:344-360.

Perlstein, Meyer A. and Ramzy Atalla. 1966. Neurologic sequelae of plumbism in children. Clinical Pediatrics 5:292-298.

Pincussen, Ludwig. 1933. Über die Beforderung der Ausscheidung von Blei durch Bestrahlung. Klinische Wochenschrift 12:275.

Plotnikoff, N., D. Reinke and J. Fitzloff. 1962. Effects of stimulants of rotarod performance of mice. Journal of Pharmaceutical Sciences 51:1007-1008.

Popoff, Nina, Sidney Weinberg and Irwin Feigin. 1963. Pathologic observations in lead encephalopathy. With special reference to the vascular changes. Neurology 13:101-112. (Abstracted in Biological Abstracts 42:21809. 1963)

Rapoport, Milton and Mitchell I. Rubin. 1941. Lead poisoning: A clinical and experimental study of the factors influencing the seasonal incidence in children. American Journal of Diseases of Children 61:245-255.

Reed, C. D. and J. A. Tolley. 1967. Lead in drinking water. The Lancet, 1967, Vol. 2, p. 1258.

Reisberg, Ruth Berman. 1954. Sulfhydryl groups of choline acetylase. Biochemica et Biophysica Acta 14:442-443.

Rezin, Paul F. and Philip Drinker. 1939. Volatilization of lead below 800°C. Journal of Industrial Hygiene and Toxicology 21: 461-463.

Robinson, J. and C. G. Hunter. 1966. Organochlorine insecticides: concentrations in human blood and adipose tissue. Archives of Environmental Health 13:558-563.

Sandstead, Harold H. 1967. Effect of chronic lead intoxication on in vivo I-131 uptake by the rat thyroid. Proceedings of the Society for Experimental Biology and Medicine 124:18-20.

Sartain, Peggy, Jo Anne Whitaker and Janet Martin. 1964. The absence of lead lines in bones of children with early lead poisoning. The American Journal of Roentgenology, Radium Therapy and Nuclear Medicine 91:597-601.

Sávay, G. and B. Csillik. 1958. Lead-reactive substances in myoneural synapses. Nature 181:1137-1138.

_____ 1959. Lead reactive substances in peripheral synapses. Experientia 15:396-397.

Schroeder, Henry A., William H. Vinton and Joseph J. Balassa. 1963. Effects of chromium, cadmium and lead on the growth and survival of rats. The Journal of Nutrition 80:48-54.

Sladek, N. E. and G. J. Mannering. 1969. Induction of drug metabolism. II. Qualitative differences in the microsomal N-demethylating systems stimulated by polycyclic hydrocarbons and by phenobarbital. Molecular Pharmacology 5:186-199.

Snedecor, George W. 1956. Statistical methods. 5th ed. Ames, Iowa, Iowa State University. 534 p.

Spector, William S. (ed.). 1956. Acute toxicities of solids, liquids, and gases to laboratory animals. Philadelphia, W. B. Saunders. 408 p. (Handbook of toxicology, Vol. 1)

Srivistava, P. C. and S. Varadi. 1968. Lead poisoning from unusual source. British Medical Journal, 1968, Vol. 1, p. 578.

Thomas, Heriberto V., Benno K. Milmore, Gerald A. Heidbreder and Benjamin A. Kogan. 1967. Blood lead of persons living near freeways. Archives of Environmental Health 15:695-702.

Thurston, Don L., J. Neal Middlekamp and Elizabeth Mason. 1955. The late effects of lead poisoning. Journal of Pediatrics 47: 413-423.

Toman, James E. P. 1951. Neuropharmacologic considerations in psychic seizures. Neurology 1:444-460.

Tschudy, Donald P., Alan Waxman and Annie Collins. 1967. Oscillations of hepatic delta-aminolevulinic acid synthetase produced by estrogen: a possible role of "rebound induction" in biological clock mechanisms. Proceedings of the National Academy of Sciences 58:1944-1948.

Vahlquist, Bo and Henry Sälde. 1943-44. Lead poisoning in growing individuals. Clinical and experimental studies. Acta Pediatrica 31:180-210. (Abstracted in Chemical Abstracts 41:3861e. 1947)

Waldron, H. A. 1964. Serum aspartate and alanine transaminase levels in workers exposed to lead. Journal of Clinical Pathology 17:149.

West, Irma. 1967. Lindane and hematologic reactions. Archives of Environmental Health 15:97-101.

Wigglesworth, V. B. The mode of action of DDT. In: DDT: the insecticide dichlorodiphenyltrichloroethane and its significance, ed. by Paul Muller. Vol. 1. Basel, Birkhauser. p. 93-111.

Wilkinson, J. Henry. 1968. Measurement of delta-aminolevulinic acid in biological fluids. In: Manual of procedures for the applied seminar on laboratory diagnosis of diseases caused by toxic agents, ed. by William F. Sunderman and William F. Sunderman, Jr. Baltimore, Association of Clinical Scientists. p. XIII-1 - XIII-5.

Williams, Huntington, Wilmer H. Schulze, H. B. Rothchild, A. S. Brown and Frank R. Smith. 1933. Lead poisoning from burning of battery casings. The Journal of the American Medical Association 100:1485-1489.

Wintrobe, Maxwell M. 1961. Clinical hematology. 5th ed. Philadelphia, Lea and Febiger. 1186 p.

Yaverbaum, P. M. 1965. Serum aldolase activity following contact with lead. Federation Proceedings, translation suppl. 24:T63-T64.

Zavon, Mitchell R., Charles H. Hine and Kenneth D. Parker. 1965. Chlorinated hydrocarbon insecticides in human body fat in the United States. The Journal of the American Medical Association 193:837-839.